Chromatography: A Perspective

James E. Meinhard

Department of Chemistry, University of Wisconsin, Madison

IN 1906 the Russian botanist, Tswett, published in the proceedings of the German Botanical Society (*30*) a description of a new technique for separating the components of complex mixtures. This technique, which was based on the partition of solutes between a stationary solid adsorbent and a moving liquid phase, was dealt with more comprehensively in a later work in Russian (*31*). It consisted simply of pouring a small quantity of the solution of pigments, such as green leaf pigments, on the top of a vertical column of adsorbent and following this with pure solvent, whereupon a series of colored bands formed down the length of the column in a sequence determined by mass relationships and adsorption coefficients. Tswett named his technique the "chromatographic method."

Unfortunately the chromatographic method got off to a bad start. In the first place, Tswett's book was published in Russian, and his original paper was published in a botanical journal, as were several later ones. Hence much of his work did not receive wide notice by chemists. In the second place, many of his publications at that time were somewhat polemic in nature. In reading the abstracts of his publications one cannot help but feel in Tswett a vigorous individualism, which probably grated on the sensibilities of the chemical hierarchy. Tswett had a clear conception of the tremendous scope of his discovery and such implicit faith in the procedure that against all opposition he insisted doggedly upon the presence of two components in chlorophyll and in the "phaeophytin" of that period. But for a lowly botanist to assault thus the whole chemical profession was unthinkable! Although a few isolated remarks appeared in favor of Tswett's work, the chromatographic method fell largely into disrepute, and, according to Zechmeister (*33*), Tswett spent the latter part of his life in misery and poverty.

Chromatography was not revived until 1931, under the stimulus of rather widespread researches on the carotenoids. In the intervening period only infrequent references to the technique can be found. Palmer, in his monograph on the carotenoids (*16*) published in 1922, speaks very highly of the technique and refers to several of Tswett's publications. The importance of the carotenoids as precursors of vitamin A was not realized, however, until 1928, when Euler and co-workers (*8*) pointed out that pure carotene, lycopin, and certain other carotenoids showed the same characteristic reaction with antimony trichloride in chloroform as did vitamin A. They further demonstrated the pronounced activity of carotenoids as growth factors and were able to show a definite relationship (*9*) between the antimony trichloride reaction, carotene content, and vitamin A potency. This new incentive led several investigators to undertake intensive studies in this field, and in 1931 Kuhn and Lederer (*13*) described the separation of carotenoids from several natural sources by "adsorption analysis" on fibrous alumina. This was followed two years later by publications by Karrer and Morf (*11*) and Karrer and Walker (*12*) describing the separation of α- and β-carotenes on calcium oxide or hydroxide. Then in 1934 two articles by Strain (*22, 23*) appeared, setting forth the excellent properties of magnesium oxide as an adsorbent for the separation of α- and β-carotenes. Strain concluded that there was no support for the view that α- and β-carotenes are artifacts arising from the action of the adsorbent. These observations appear to have broken the back of any remaining opposition to the method initiated by Tswett, and numerous publications continued to flow from the pens of Karrer and co-workers, Strain, and others.

From the early 1930's to the present day, chromatography has continued to develop in a multiplicity of directions, aided by numerous ingenious devices and refinements in technique, so that there is no longer a single branch of chemistry that has not profitably employed this separational procedure. In the words of Paul Karrer in his lecture at the International Congress of Pure and Applied Chemistry (London, 1947), quoted recently by Tiselius (*29*):

> No other discovery has exerted such a great influence and widened the field of investigation of the organic chemist as much as Tswett's chromatographic analysis. Research in the field of vitamins and hormones, carotenoids, and numerous other natural components could never have progressed so rapidly and achieved such great results if it had not been for this new method, which has also disclosed the enormous variety of closely related compounds in nature.

It is beyond the scope of this paper to treat individually of each important development or useful vari-

ation in the method. An excellent review of the more important developments is given in a recent publication by Strain (*25*). A general perspective of the scope of modern chromatography, as well as some insight into possible future developments, may be gained, however, by considering the process from the standpoint of competing equilibria and by making a classification on this basis. Such a classification assumes complete reversibility in the interactions taking place—a condition occasionally not fulfilled and, for certain applications, not always necessary. But in the general case the stationary adsorbent and the mobile fluid (which may be either gaseous or liquid) compete with each other for the solutes originally present in the fluid phase. Such a classification is given in Table 1.

TABLE 1

Primary mode of action	Class	Type of competitive interaction: Mobile phase	Type of competitive interaction: Solid phase	Representative phenomena*
Ionic: (solubility product, instability constant, etc.)	1	Ions	Ions	Ion exchange
	2	Complex	Ions	Ion exchange
	3	Ions	Complex	Inorganic chromatography
	4	Complex	Complex	(Not reported)
	5	Ions	Dispersion	Fractional decomposition methods
	6	Complex	Dispersion	Fractional decomposition methods
	7	Dispersion	Dispersion	Diffusion of colloids, macromolecules
	8	Ions	Association	Electrokinetics
	9	Dispersion	Ions	(Not reported)
	10	Dispersion	Complex	(Not reported)
Nonionic: H-bonding; dipole, and/or induced dipole; geometry of molecules (partition coefficients)	11	Association	Association	Organic (and partition) chromatography
	12	Association	Dispersion	Catalytic polymerization
	13	Association	Ions	Catalytic dissociation
	14	Association	Complex	(Not reported)
	15	Complex	Association	(Not reported)
	16	Dispersion	Association	Salting-out adsorption

* The items in this column are admittedly incomplete and dependent primarily on the author's limited knowledge. The reader may be able to suggest phenomena that could be included in the spaces labeled "not reported."

In this table the following definitions apply:

Ion: A solute particle having a definite electrical charge and characterized by a simple structure and subcolloidal dimensions.

Complex: The product of a reversible interaction between a solute particle of simple structure and a complexing agent to form a new species. The combination usually involves linkages of an auxiliary, or coordinate, character. The complex may itself be a simple structure (charged or uncharged), or it may be a relatively large and complicated aggregation of particles appearing as a colloidal dispersion, as a precipitate, or as an immobile deposit on the solid phase.

Dispersion: The product of a reversible interaction of a solute with another solute, or with the solvent itself, to produce a species having a decreased association with the solvent. This decrease in association becomes evident in the aggregation of particles to form a colloidal dispersion or a visible precipitate. In this discussion the term dispersion is not intended to include those dispersons arising from a process of complexing as described in the preceding paragraph. It does, however, include dispersions of proteins, silicates, soaps, dyes, organic polymers, and other particles which may or may not be ionically charged, depending upon pH, adsorption or occlusion of ions, presence of dissociable groups, etc.

Association: A mutual attraction between a solute particle and another substance (either in the liquid or solid phase, or both), or between two solute particles of the same species, which does not involve the establishment of fixed bonds. This interaction is characterized, rather, as a loosely bound complex of indeterminate composition arising from the geometry of the molecules involved, from dipole and induced dipole interactions, from resonating electrostatic attractions, or from other imperfectly understood factors. The "bond energies" involved, insofar as this term may be applied here, lie in the neighborhood of 2 to 8 kilocalories per mole, as contrasted with the much greater bond energies involved in the types of aggregation discussed previously.

A casual study of these types of interaction will reveal that the selection is to some extent arbitrary. It is difficult or impossible to assign quantitative definitions to these terms. Yet the phenomena they represent are real, and their selection is based on their suggestiveness of these phenomena in accordance with current usage. Consequently, they are employed here as descriptive titles rather than as terms of exact definition. As such, they are paired in Table 1 to

form sixteen possible combinations that theoretically may give rise to competing equilibria by means of their relationships to the mobile phase and the solid phase. The title gives the key to the type of relationship existing. Each pair describes a class. Several of the classes represented will be recognized as already popular in chromatographic methods. Others are just being introduced, or have not as yet been suggested. It is the purpose of the following paragraphs to consider briefly the development and prospects of chromatography as related to these classes.

Class 1: Chromatography by ion exchange has been widely studied. Schwab and co-workers have done considerable pioneering in this field (*19, 20*) and have applied inorganic chromatography as an adjunct in microanalysis (*18*). Perhaps one of the most impressive applications of this method is the concentration of the isotopes of potassium, lithium, and nitrogen by Taylor and Urey (*27, 28*) using columns of zeolites. The relative adsorption affinities of ions appear to depend primarily on valence, degree of hydration, and basicity. The separation of zirconium and hafnium on an ion exchange resin has been announced recently (*26*), development being carried out with 6 M hydrochloric acid. The more basic hafnium appears first in the effluent.

Class 2: An important refinement of simple ion exchange chromatography is described in considerable detail by Spedding, Boyd, Tompkins, and others (*21*). These investigators employed buffered citrate solutions in the chromatographic development of rare earth mixtures on synthetic ion exchange resins. The competition between resin and complexing agent for the individual elements gives rise to a separation depending mainly on relative basicities. It was found possible to separate in a comparatively small number of operations various mixtures of rare earth elements into spectroscopically pure individuals. To obtain a similar result by older methods requires as much as a thousand fractional recrystallizations. The chromatography of fission product mixtures has led to the first positive chemical identification of isotopes of element 61 (*14*).

Class 3: By reversing the system described as Class 2 and selecting a complexing agent for the solid phase it is again possible to achieve separation of ions. Erlenmeyer and Dahn (*7*) have pioneered in this method, employing 8-hydroxyquinoline as the "adsorbent." The position of several metals are recognizable on the column by the colored complexes they form. The pH of the liquid phase is an important factor, since the stabilities of the complexes are directly related to it. The use of this method does not seem to be widespread, possibly because of the limited number of complexing agents possessing favorable characteristics and also because even 8-hydroxyquinoline itself tends to be displaced from the solid phase during the process of development. It would seem that these difficulties could be overcome by "fixing" the complexing agent irreversibly on another solid. Silica, for example, might be precipitated in the presence of 8-hydroxyquinoline to form an "oxinated" silica with the desired characteristics. Much developmental work is obviously possible.

Class 4: Finally, competing equilibria may be established between a complex in the mobile phase and a complex in the solid phase. A mixture of citrate complexes, for example, might be chromatographed on a column of 8-hydroxyquinoline. Here again, the separation will depend on the basicity of the complexed ion, and the sequence of zones should be predictable on the basis of ionic crystal radii. While this method increases the separational possibilities included under the first three classes, no work seems to have been done on it.

Class 5: A solution of cations by chromatographic development may be induced to precipitate in colloidal form through a gradual increase in pH. The colloidal material, being less mobile than the original ions, is retained in the interstices of the adsorbent as a "zone." This mechanism has been pointed out recently by Meinhard and Hall (*15*), and it is felt that some of the previously reported inorganic separations regarded as proceeding by simple ion exchange in reality occur by this process. The method is limited at present primarily to analytical applications on a micro or semimicro scale. Its success will depend on refinements in technique which lead to reliable quantitative estimations.

Class 6: The system described as Class 5 may be modified by first complexing the original ions. The mechanism will then involve decomposition of the complex prior to the precipitation of a zone. Separations will be based on the relative stabilities of complexes and the mobility of the complexing agent. This system should not be confused with Class 15, in which the complex is adsorbed as such on the column. Where the chromatography of simple ions does not lead to a useful separation, a complexing agent may be employed to modify both the sequence and the sharpness of definition of the zones. Little work appears to have been done on this type of separation (*10*) and the formation of a less mobile species by hydrolysis seems to have been largely overlooked.

Class 7: Chromatographic methods so far do not seem to have found much application in the separation of proteins and other macromolecules or molecular aggregates. In this classification the extent of ag-

gregation of the colloid in the liquid phase is often different from, and usually less than, its aggregation when in contact with the solid adsorbent. Unfortunately, proteins are often irreversibly adsorbed and may suffer denaturation at the solid-liquid interface. Adsorption studies on enzymes initiated by Willstätter (*32*), and his school, have resulted in certain successful separations on alumina and silica gel, but such adsorbents cannot be applied generally. An obvious suggestion here, and one that apparently has not been reported, is the chromatography of protein dispersions using as the immobile phase a solid protein structure. Such a structure would seem to be capable of a maximum degree of specificity combined with a maximum degree of "gentleness" toward the adsorbate.

A somewhat different application of this method has been employed recently in the purification of zirconyl salts (*1*). This procedure depends on the formation of the hydrous oxide of zirconium when zirconyl nitrate is dissolved in water at moderate concentrations. The hydrous oxide exists in colloidal form and is nonionic. By passing the solution through an ion exchange column all ions existing as impurities are retained, while the zirconyl colloid appears quantitatively in the effluent. A similar process has been indicated (*4*) for the commercial preparation of silica dispersions. A solution of sodium silicate is developed on an acid-treated ion exchange resin, which retains the sodium ion and releases a salt-free colloidal silicic acid.

Class 8: When a solid does not contain individual ionic centers of attraction its activity toward the passage of a solution of ions is manifested in the electrokinetic effect. Here the ions are associated with the solid in a postulated electric double layer. A recent description of the process is given by Bikerman (*2*). The effect becomes prominent only at very low concentrations and, in the case of uni-univalent electrolytes, is proportional to the concentration$^{-1/2}$. It is difficult to see how such a system could be made the basis of a useful chromatographic method, especially in view of the high potency of ion exchange media. It is interesting, however, to consider this effect in relation to the migration of ions in soils and other geological formations.

Class 9: The chromatography of a colloidal dispersion which combines ionically with the adsorbent has been suggested for proteins using an ion exchange resin (*29*). The reaction of the colloid with ion exchange media might be predictable on the basis of its interaction with other ionic species. It is impossible, however, to assess the potential value of such a method in the absence of experimental data. An interesting possibility is present in the case of insoluble inorganic salts. If an aqueous dispersion of barium sulfate, for example, is chromatographed on an acid-treated cation exchange resin, the barium ion should be adsorbed while sulfuric acid appears in the effluent. Development of the column may be continued using a different acid, the net result being a barium salt whose original anion has been replaced. The chromatographic separation thus accomplished is one of cations from anions. A separation of a mixture of cations obviously can take place at the same time. Such an application should find use in certain analytical schemes where sulfate, or other anions, are objectionable and difficult to eliminate.

Class 10: If for the ion exchange resin of Class 9 a complexing agent is substituted, still another chromatographic system is obtained. If it is desired to chromatograph on 8-hydroxyquinoline a mixture of metals obtained as difficultly soluble salts, one may add them as a dispersion directly to the column rather than resorting to additional operations designed to convert them to a more soluble form. Using a buffer solution as the developing solvent, the original anions may be carried into the effluent and the metal zones themselves may be separated. Such a method, of course, has only a limited usefulness.

Class 11: Chromatography owes its present popularity primarily to its tremendous success in the field of organic chemistry. In this classification the usurping effects of ionic charges are present to a negligible degree only, and separations become dependent on the associations of the solutes with solvent and adsorbent. Since differences in degree of association may arise from minute structural alterations, separations are based on structure alone. The separation of *cis*- and *trans*-isomers, and of optically active isomers, eloquently demonstrates the potency of this method. One of the foremost publications on chromatography, especially with respect to organic separations, is the book by Zechmeister and Cholnoky: *Die chromatographische Adsorptionsmethode* (*34*). In spite of the extensive studies made in this realm, chromatography still remains largely an art rather than a science (*24*).

Partition chromatography also may be placed in this category. Here the "solid" phase is actually a stationary immiscible liquid phase, and the rate of migration of the solute is a function of its partition coefficient in the two liquids. The filter paper chromatography of protein hydrolyzates initiated by Consden, Gordon, and Martin (*3*) is an example of this method that has attained widespread use. It should be pointed out, however, that the partition chromatography mechanism is valid in a more general sense. It is generally agreed from independent experiments that retention of liquid at a solid-liquid interface may ex-

tend to a depth of as much as a hundred molecules or more, provided the solid is "wetted" by the liquid. In viscous flow such a layer may be regarded as a "stationary" phase. Further, the structure of the stationary liquid is distinctly modified by the proximity of the adsorbent, thus completing the resemblance to an immiscible liquid phase. It will be seen, therefore, that the difference between ordinary chromatography and partition chromatography depends primarily on the selection of media and not on the mechanism involved.

Class 12: The catalytic effect of an adsorbent on a given solute molecule occasionally results in polymerization to a species which forms colloidal aggregates. This material may be adsorbed irreversibly or it may wander down the column at a sufficiently modified rate that a separation may be effected from another solute not similarly acted upon. The use of such a method is distinctly limited, especially since the polymerization process is in most cases irreversible.

Class 13: An adsorbent may also cause catalytic dissociation of a solute molecule with the formation of an ionic species in the adsorbate. Here again the process may be irreversible. By development with a sufficiently polar solvent, however, recombination of ions may occur, resulting in an equilibrium process. Obviously, in certain cases such a recombination may give rise to a more complex mixture than the original material. The process has been made the basis of various separations, however, in certain types of partition chromatography where a solvolytic ionization takes place in the aqueous phase. Elsden (*5*), for example, has separated mixtures of fatty acids in chloroform on a silica gel column, using bromcresol green as a column indicator.

Class 14: The employment of a solid phase which forms a complex with an organic solute does not appear to have been reported. Picric acid is suggested here as a complexing agent which might be used as the "adsorbent." Since it is soluble in organic solvents, it must first be rendered immobile either by the introduction of hydrophilic groups into the molecule or by irreversible adsorption on a suitable solid. Such a modified adsorbent may then be used in the chromatography of aromatic hydrocarbons, ethers, etc., the separation depending upon the relative stabilities of the corresponding picrates. Inasmuch as such picrates are often highly colored, the progress of a zone down the column may be readily traced. Other similar complexing agents include *sym*-trinitrobenzene, picrolonic acid, styphnic acid. Mercury salts might be used in the chromatography of certain unsaturated compounds; and such materials as calcium chloride and aluminum chloride, could be used in the separation of aliphatic ethers. For the separation of halogen derivatives, the use of ion exchange resins containing amine and imine groups is possible. All of these systems require essentially anhydrous conditions in order to avoid complications of an ionic character.

Class 15: The solubility of various metal complexes, such as the dithizonates, in organic solvents has led to a number of extraction procedures of analytical value. Little has been done, however, with the chromatography of such solutions. The separation of heavy metal dithizonates on alumina has been reported (*6*) as partially successful, but the use of other complexes in this manner has not been described. It is felt that further investigation of this method should be rewarded by useful separations which are especially applicable in trace-metal analysis.

In this same category partition chromatography is also possible. Such a system would involve the percolation of an organic solution of complexes through a column of silica gel, or other suitable water-retentive adsorbent. It might be suggested that this method be included in Class 4 except that here we are concerned primarily with the association of complexes rather than the competition between two different complexing agents. These systems may be reversed by employing an organic solvent in the solid phase and using an aqueous solution of complexes for the mobile phase.

The chromatography of aqueous solutions of metal complexes on hydrophilic adsorbents which have been pretreated with the complexing agent has been described (*35*). Where these complexes are ionic in character (e.g., the metal ammine complexes) this system becomes one of simple ion exchange and properly belongs in Class 1; where they are uncharged (e.g., soluble chelate complexes) the migration of the complex is governed primarily by its association with the adsorbent and may be included here. The developing solvent in these cases also contains the complexing agent, and the separation of zones results from a competition between the aqueous phase and the adsorbent for the complexed material. That is, dissociation of the complex itself is not a material factor in the process, as it is in Classes 2, 3, and 4. These fields of application are untouched.

Class 16: The chromatography of colloidal dispersions is beset with a number of difficulties, including the possibility of the presence of an almost limitless number of molecular species (e.g., polymers) and of widely varying degrees of aggregation. A phenomenon has been suggested recently by Tiselius (*29*), however, which promises to assist materially in the chromatography of proteins, namely, "salting-out" adsorption. Proteins of low adsorption affinity may be strongly bound to the solid phase in the presence of

a salting-out agent such as ammonium sulfate. The exact nature of the association of the protein with the adsorbent is not known, but where the strength of association varies with different proteins the basis of a chromatographic separation is established. The elution of separate proteins may be carried out simply by varying the salt concentration in the developing solvent. The success of the method requires, of course, complete reversibility among the species present.

Some progress in the chromatography of particulates ranging from virus to bacterial size has been made, and its application has been demonstrated recently (*17*) in the separation of certain subcellular, enzymatically active granules. This separation was accomplished on columns of Celite, using aqueous solutions of sodium chloride as developing solvents. The mechanism here, also, is apparently one of salting-out adsorption, inasmuch as the original adsorption was accomplished under the influence of a much higher salt concentration than was used in the development of the column. Obviously, such a procedure is of value in amplifying, or supplanting, centrifugal methods of separation.

It will be apparent to investigators familiar with chromatographic methods that the scheme advanced above is somewhat oversimplified. It often happens, for example, that two or more of the types of interaction given here occur simultaneously in varying degrees on a single chromatogram. Conversely, because the exact nature and properties of certain solute particles are not well understood, it becomes difficult to place their chromatographic behavior in a specific class. It is also apparent that many of the significant advances in instrumentation have been omitted. For a description of these, the reader is referred to the several excellent reviews and monographs on the subject.

This tabulation is designed primarily to fulfill two purposes. It is intended, first, to point out those directions in which the greatest advances in chromatography have taken place and to reveal some of the reasons for these advances. Second, it is intended to suggest other applications which have not been tried, or which have been only briefly examined, with the hope of indicating some probable areas of future research.

References

1. Ayers, J. A. *J. Amer. chem. Soc.*, 1947, **69**, 2879.
2. Bikerman, J. J. *Surface chemistry for industrial research.* New York: Academic Press, Inc., 1948, pp. 399–404.
3. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.*, 1944, **38**, 224.
4. DuPont Company. *Chem Eng. News*, 1947, **25**, 3168.
5. Elsden, S. R. *Biochem. J.*, 1946, **40**, 249.
6. Erämetsä, O. *Suomen Kemistilehti*, 1943, **16B**, 13; *C. A.*, 1946, **40**, 4620.
7. Erlenmeyer, H. and Dahn, H. *Helv. Chim. Acta*, 1939, **22**, 1369.
8. Euler, B. v., Euler, H. v., and Hellström, H. *Biochem. Z.*, 1928, **203**, 370.
9. ———. *Svensk Kem. Tid.*, 1928, **40**, 256.
10. Flood, H. and Smedsaas, A. *Tids. kjemi, Bergvesen Met.*, 1942, **2**, 17; *Chem. Zentr.*, 1942, **2**, 1269.
11. Karrer, P. and Morf, R. *Helv. Chim. Acta*, 1933, **16**, 625.
12. Karrer, P. and Walker, O. *Helv. Chim. Acta*, 1933, **16**, 641.
13. Kuhn, R. and Lederer, E. *Ber.*, 1931, **64**, 1349.
14. Marinsky, J. A., Glendenin, L. E., and Coryell, C. D. *J. Amer. chem. Soc.*, 1947, **69**. 2781.
15. Meinhard, J. E. and Hall, N. F. *Anal. Chem.*, 1949, **21**, 185.
16. Palmer, L. S. *Carotenoids and related pigments.* New York: Chemical Catalog Company, 1922.
17. Riley, V. T. *et al.* *Science*, 1949, **109**, 361.
18. Schwab, G. M. and Ghosh, A. N. *Angew. Chem.*, 1939, **52**, 666.
19. Schwab, G. M. and Jockers, K. *Naturwissenschaften*, 1937, **25**, 44.
20. ———. *Angew. Chem.*, 1937, **50**, 546.
21. Spedding, F. H. *et al.* *J. Amer. chem. Soc.*, 1947, **69**, 2769.
22. Strain, H. H. *Science*, 1934, **79**, 325.
23. ———. *J. biol. Chem.*, 1934, **105**, 523.
24. ———. *Ind. Eng. Chem.*, Anal. Ed., 1942, **14**, 245.
25. ———. *Anal. Chem.*, 1949, **21**, 75.
26. Street, K., Jr. and Seaborg, G. T. *J. Amer. chem. Soc.*, 1948, **70**, 4268.
27. Taylor, T. I. and Urey, H. C. *J. Chem. Phys.*, 1937, **5**, 597.
28. *Ibid.*, 1938, **6**, 429.
29. Tiselius, A. *Chem. Eng. News*, 1949, **27**, 1041.
30. Tswett, M. *Ber. deut. botan Ges.*, 1906, **24**, 384.
31. ———. *Chromophylls in plant and animal world.* Warsaw. Warshawskago utschebnago Okruga, 1910.
32. Willstätter, R. *Untersuchungen uber Enzyme.* Vol. I, p. 66. Berlin: Julius Springer, 1928.
33. Zechmeister, L. Address to Wisconsin Section, A.C.S., 1946.
34. Zechmeister, L. and Cholnoky, L. v. *Principles and practice of chromatography.* New York: John Wiley, 1941.
35. *Ibid.*, p. 312.

TECHNICAL PAPERS

The Proteins of Mammalian Spermatozoa and Cellular Nuclei[1]

Lloyd E. Thomas and Dennis T. Mayer

Department of Biochemistry, School of Medicine and *Department of Agricultural Chemistry, College of Agriculture, University of Missouri, Columbia*

Since the early work of Miescher, Kossel, and others, cellular nuclei and fish spermatozoa have been known to contain a basic protein (either histone or protamine) and nucleic acid. Miescher (*8*) and Mathews (*3*) were unable to demonstrate the presence of histones or protamines in bull spermatozoa.

Mayer and Gulick (*4*) separated thymus nuclei by using Behrens' technique and extracted from the nuclei, by means of warm 3% NaOH solution, a protein fraction which precipitated when the solution was brought to about pH 6. They also extracted another fraction from thymus nuclei with a warm 5% NaCl solution. This material was precipitated by dialysis.

Mirsky and Pollister (*5, 6*) used 1 M NaCl to extract from minced tissues a nuclear material which was precipitated by dialysis or dilution. In addition to histone and nucleic acid, the extracts contained in suspension a tryptophane-containing protein fraction. They were unable, however, to extract this material from bull spermatozoa (*6*). Mirsky and Ris (*7*) later identified a "residual protein," obtained from isolated chromosomes, with this tryptophane-containing protein fraction. The residual protein was insoluble in any medium which leaves proteins intact.

Stedman and Stedman (*9*) found a protein fraction other than histone in cellular nuclei. This protein fraction, which they called "chromosomin," was reported (*10*) to be insoluble in dilute acids and alkalies. It required from 1 to 3 days for dissolution in 1.0 N NaOH solution. The "chromosomin" from cod spermatozoa was found to contain 9.8% arginine, and the same fraction from Walker rat sarcoma 8.0% arginine (*11*).

Green (*2*) extracted ram spermatozoa with dilute acid and alkali, and suggested that the residue was probably the membrane substance. Analysis of the residue showed that it contained no lysine, approximately 23% arginine, and 13% histidine.

The following summarizes the results of rather extensive work by the authors on boar and ram spermatozoa. Most of this work was done on boar spermatozoa.

Our work indicates that neither boar nor ram spermatozoa contain any material soluble in distilled water or 1.0 or 2.0 M NaCl solutions. Grinding of the boar spermatozoa or freezing them to −40° C, and thawing, did not affect the results with the reagents mentioned. Dilute or concentrated acids, dilute alkalies, detergent solutions, and thioglycollic acid solution extracted only a small amount of material from these cells.

Extraction by stirring for 30 min at room temperature with 1.0 N NaOH, however, removed two protein fractions from boar spermatozoa which precipitate at about pH 6.0 and pH 4.5 respectively. At this point most of the tails and midpieces had disappeared, but the heads retained their shape. The pH 6.0 fraction is so large that it can hardly have come from the tails and midpieces. It contained less than 1% phosphorus. The following amino acids were found in hydrolyzates of this fraction by means of paper chromatography:[2] arginine, lysine, histidine, proline, valine, leucine (isoleucine), phenylalanine, tyrosine, methionine, alanine, threonine, serine, glycine, cystine, glutamic acid, aspartic acid, and two unidentified substances. Microbiological assay[3] showed that tryptophane is also present. A chemical method and a microbiological method both showed the pH 6 fraction to have an arginine content of about 10%.

The smaller pH 4.5 fraction may consist of some of the pH 6.0 fraction, combined with a small amount of nucleic acid.

The residual material left after a half-hour extraction with 1.0 N NaOH contains most of the nucleic acid of the cells. Paper chromatography of hydrolyzates of this material showed the presence of arginine, proline, valine, leucine (isoleucine), phenylalanine, alanine, threonine, serine, glycine, cystine, glutamic acid, and aspartic acid. Microbiological assay indicated that tryptophane is also present. Neither method showed more than traces of methionine, histidine, or lysine. Both chemical and microbiological determinations indicated the presence of a large quantity of arginine. Calculations allowing for the nucleic acid content of this fraction indicated that the arginine content of the protein part is at least 25%.

Allowing this residual material to stand overnight at room temperature with 1.0 N NaOH brings into solution some material which contains protein but is predominantly nucleic acid. The dissolved material precipitates at about pH 2–3 when the solution is acidified. It seems that the long treatment with alkali probably brings about the splitting of a nucleoprotein complex. Practically nothing is brought into solution when the long treatment with alkali is carried out at 5° C. The portion still undissolved after this long alkali treatment contains what appear to be "ghost" heads of spermatozoa and also some fine granular material.

[1] Contribution from the Department of Agricultural Chemistry, Missouri Agricultural Experiment Stations, Journal Series No. 1157.

[2] The paper chromatography was kindly done for us by Dr. Eugene Roberts, of the Cancer Research Division of the Anatomy Department, Washington University School of Medicine, St. Louis.

[3] We are indebted to Mr. A. Lee Caldwell, of Eli Lilly and Company for the microbiological assays.

Previous work by other investigators and our work would seem to indicate that nuclei, in general, contain the following:

1. Nucleohistones or nucleoprotamines extractable with water or NaCl solutions.

The mammalian spermatozoa so far studied differ from other nucleated cells in containing no substances soluble in water or NaCl. These substances may be present in an altered form with different solubility characteristics.

2. Proteins extractable with alkali and precipitating when the solution is made acid.

The pH 6 fraction from thymus nuclei (Mayer and Gulick) and the pH 6 and 4.5 fractions from boar spermatozoa are protein fractions of this nature. This type of protein can be included only provisionally as a constituent of nuclei in general.

3. A highly insoluble residual material containing proteins and nucleic acids.

Green's residue from ram spermatozoa, the tryptophane-containing protein of Mirsky and Pollister, the "residual chromosome" of Mirsky and Ris, the "chromosomin" of Stedman and Stedman, and our residual material from boar spermatozoa all seem to belong in this category. They are all highly insoluble and require drastic treatment to bring even a part into solution.

A highly significant characteristic of the residue obtained by Green from ram spermatozoa and of our residue from boar spermatozoa is the high arginine content. The absence of lysine from these residues is also of interest.

Davidson and Lawrie recently reported (*1*) the results of amino acid analysis by paper chromatography of histone and residual material from calf thymus, rat liver, and fowl erythrocyte nuclei. These authors noted the absence of lysine in the residual material from all three of these sources. The quantity of arginine present was not reported.

The presence of more than one type of protein in each of these insoluble residues must be considered. The proteins with high arginine content in some of these residues may be, as suggested, altered forms of nucleohistones. On the other hand, the proteins of high arginine content in these residues may represent an entirely different type of protein.

References

1. Davidson, J. N. and Lawrie, R. W. *Biochem. J.*, 1948, **43**, XXIX.
2. Green, W. W. *Anat. Rec.*, 1940, **76**, 455.
3. Mathews, A. *Z. physiol. Chem.*, 1897, **23**, 399.
4. Mayer, Dennis T. and Gulick, Addison. *J. biol. Chem.*, 1942, **146**, 433.
5. Mirsky, A. E. and Pollister, A. W. *Proc. nat. Acad. Sci., Wash.*, 1942, **28**, 344.
6. ———. *J. gen. Physiol.*, 1946, **30**, 117.
7. Mirsky, A. E. and Ris, Hans. *J. gen. Physiol.*, 1947, **31**, 7.
8. Schmiedeberg, O. (Ed.). *Die histochemischen und physiologischen arbeiten von Friedrich Miescher.* Leipzig: von F. C. W. Vogel, 1897.
9. Stedman, E. and Stedman, E. *Nature*, Lond., 1943, **152**, 267.
10. ———. *Sympos. quant. Biol.*, 1947, **12**, 224.
11. ———. *Sympos. Soc. exp. Biol.*, 1947, **1**, 232.

A Possible Standard for Radioiodine

A. H. W. Aten, Jr.

Institute for Nuclear Research, Amsterdam, Netherlands

Recently a radioactive thallium isotope has been suggested as a standard for comparison with I^{131} (*1*). It seems likely, however, that Cl^{36} will be more satisfactory for the purpose. Its maximum β-energy, 0.66 mev, differs very little from the corresponding value for I^{131}, 0.60 mev. Radioiodine has a second limit of minor importance at about half this value (*3*, *4*).

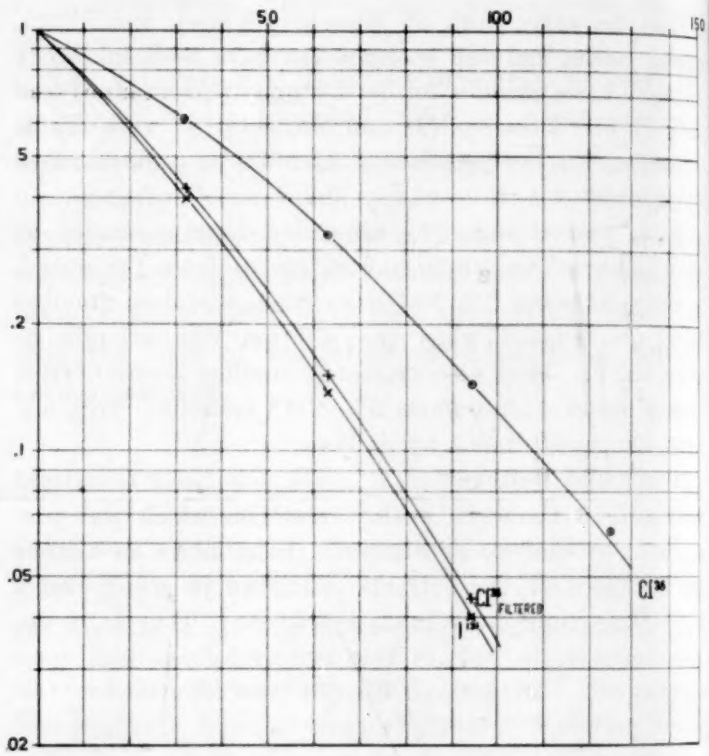

FIG. 1. Absorption of the beta rays of Cl^{36} and I^{131} in aluminum. The curve +++ indicates beta particles already filtered through 125 mg/cm². It is the continuation of the top curve.

The difference between the β energies is, however, sufficient to make the absorption curves quite dissimilar (Fig. 1).[1] As both lines curve downward in the normal way, it is possible to select two parts, one for each line, which have the same direction. Thus the absorption of Cl^{36} β particles, which have passed through 125 mg/cm² of aluminum, coincides almost exactly with the absorption of the unfiltered β radiation of I^{131}.[2] This suggests the use of a preparation containing Cl^{36} covered with 125 mg/cm² of aluminum as a standard for I^{131}. As only weak gamma rays are emitted by Cl^{36} (*2*) there is no ob-

[1] The absorption of the β radiation of Cl^{36} was determined in connection with measurements performed for Mr. C. B. Heyn. The Cl^{36} had been allotted to him for physiological investigations by the Atomic Energy Commission. The radioiodine had been furnished by the Isotope Branch of the Atomic Energy Research Establishment, Harwell.

[2] Radiochlorine had been purified from radiophosphorus and radiosulfur. Further purification caused no change in the absorption curve. The absorption of radioiodine beta rays was checked by comparison with a second preparation, obtained a few weeks earlier.

jection to the reduction of the intensity caused by this filtering.

A Cl^{36} sample, prepared as described, was compared with a radioiodine sample, using different counters which happened to be readily available in our institute. The results are seen in Table 1.

TABLE 1

Counter type	Window thickness mg/cm²	Ratio of cpm with standard and with radioiodine
Tracerlab	2.73 (mica)	1.08
Beta counter	1.5 (mica)	1.06
X ray counter	4.3 (mica)	1.06
Philips Eindhoven	about 80 (steel tube)	1.41

It is seen that the agreement with mica window counters is excellent, and even with a steel tube counter, a type of instrument which will never be chosen for standardizing radioiodine, the agreement is not worse than the average result obtained in different laboratories (*1*). This means that, with our standard, radioiodine samples can be counted under different counting arrangements without corrections.

The standard should not be used at a distance much less than half the diameter of the counter's sensitive surface. Results are best between this distance and twice its value; if larger variations are allowed results tend to be less satisfactory and deviations of about 25% may occur.

References

1. Feitelberg, Sergei. *Science*, 1949, **109**, 456.
2. Johnston, F. and Willard, J. E. *Phys. Rev.*, 1949, **75**, 528.
3. Metzger, F. and Deutsch, M. *Phys. Rev.*, 1948, **74**, 1640.
4. Zaffarano, D. J., Mitchell, A. C. G., and Kern, B. D. *Phys. Rev.*, 1949, **75**, 1632.

The Inhibition of the Cholinesterase Activity of Human Blood Plasma by Neutral Phosphate Esters. II: Studies with Hexa 1-C^{14}-Ethyl Tetrapolyphosphate[1]

Ralph W. Brauer and Rita L. Pessotti

Department of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State University, New Orleans

Compounds containing the grouping P-O-R (R = alkyl or aryl) are potent inhibitors of human plasma cholinesterase if they also include a sterically strained configuration around the phosphorus atom (as in tri *o*-cresyl phosphate), or if that atom participates in an acid anhydride linkage (as in the case of the tetraalykl pryophosphates) (*2*). The reaction between enzyme and inhibitor results in the rapid inactivation not only of the enzyme, but also of the inhibitor. Inactivation of the inhibitor is observed only on reaction with active esterase, and inactivation of the enzyme does not take place if the pyrophosphate linkages of compounds like tetraethyl pyrophosphate have been hydrolyzed by even brief contact of the inhibitor with water. The inhibition of plasma cholinesterase activity by such compounds is not reversed on removal of the inhibitor by hydrolysis and prolonged dialysis.

These observations strongly suggest that the interaction of plasma cholinesterase with such phosphorus compounds results in the formation of rather stable compounds involving the active groupings of the enzyme.

TABLE 1

Specific activity of HETP	7.02×10^2 cpm/mM
Activity added as HETP to all preparations	5.74×10^3 cpm/mg protein
Activity recovered in protein precipitated after dialysis:	
From active enzyme + intact HETP	8.05 cpm/mg protein
From active enzyme + hydrolyzed HETP	1.43 cpm/mg protein
From denatured enzyme + intact HETP	1.03 cpm/mg protein

In order to further test this hypothesis, hexaethyl tetrapolyphosphate (HETP) containing P^{32} or C^{14} as tracer atom has been prepared. An earlier test employing P^{32} tagged material (*2*) merely demonstrated that less than 1.0% of the HETP added was fixed on the enzyme, suggesting that the anticholinesterase activity of this material might reside in a minor component obtained in the course of preparation; possibly this component could be tetraethyl pyrophosphate (TEPP), the most active member of this group detected so far. The purpose of the present note is to report results obtained under more favorable conditions involving the use of C^{14}-labeled HETP.

The preparation of the inhibitor from P_2O_5 and $P(O)(OC_2H_5)_3$ was carried out as described previously (*2*), except that C^{14}-containing triethyl phosphate, prepared by reacting $CH_3C^{14}H_2I$ with silver phosphate, was employed.[2] The preparation obtained had a specific activity of 3 mc/mM of hexaethyl tetraphosphate. The human plasma esterase preparation employed[3] had a cholinesterase activity of 5.24 M CO_2 evolved/hr/g under standard conditions (0.8 M acetyl choline bromide) (*1*); this preparation is approximately ten times as pure as the fraction IV-6-3 previously employed.

The plan of the experiments, based upon these methods may be seen from Table 1. The following are additional details of preparation: a slight excess of inhibitor, or its equivalent, was added to the enzyme; HETP was hydrolyzed by contact with water (1% solution) for 72

[1] Supported by a grant from the Division of Research Grants and Fellowships, U. S. Public Health Service.

[2] This preparation was carried out for this laboratory by Tracerlab, Inc., Boston, Massachusetts.

[3] This material was made available through the kindness of Dr. Surgenor, Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts.

hr; less than 0.1% of the initial anticholinesterase activity remained at this time. Denatured enzyme was prepared by heating the enzyme solution to 80° C for 20 min to complete inactivation; 0.45% concentrations of protein were used in all cases. Dialyses were carried out in a rotating rack against 15 successive changes, at 8-hr intervals of 0.3% NaCl (30 ml per 4 ml enzyme solution each time); no C^{14} could be detected in any dialyzates after the tenth change. Precipitation of the protein from the dialyzed solutions was performed by the addition of five volumes of acetone to one of enzyme solution, after preliminary trial had shown that results obtained by this procedure did not differ from those obtained under the much more elaborate conditions required to obtain a dry precipitate of native protein. For counting, samples weighing 2.8–3.1 mg were collected on filter paper disks of 3.9 cm^2, dried in a vacuum desiccator, and counted directly (2.0 mg/cm^2 end window counter). Since all samples fell within the same narrow and low range of mass thickness, no self-absorption correction was applied. A standard was prepared by combustion of a known weight of HETP, collection of the CO_2 formed as $BaCO_3$, dilution of the activity with a known proportion of $BaCO_3$ in a homogenizer, and counting of a 3.0-mg sample on filter paper.

Three experiments carried out by these procedures yielded results in close agreement. Table 1 shows the averages of the values obtained. Three points are immediately evident from inspection of the figures. There is a definite, measurable uptake of C^{14} by the enzyme on reaction with active inhibitor. There is much less, though yet measurable, protein fixation of C^{14} under conditions which do not result in enzymologically detectable reactions between protein and phosphorus compounds. Only a small fraction of the HETP required to inhibit the enzyme is actually bound by the protein.

From the figures for C^{14} uptake and for the specific activity of the sample of HETP employed, molecular relations can be calculated if the molecular weight of the protein is known. Taking 3×10^5 as, at present, the most likely value for the mean molecular weight of this protein, a figure of 3.45×10^{-2} mol of HETP bound per mol of protein is obtained.

Assuming that inhibition of 1 mol of enzyme involves the firm fixation of 1 mol of inhibitor, these data suggest that the protein preparation employed actually contained about 3% active enzyme. This estimate is in good accord with values derived from other considerations.

The protein-to-inhibitor ratio derived from the fixation of C^{14} is also in good accord with the ratio calculated for TEPP from data on enzyme inhibition. In this case, 50% inhibition of the present enzyme preparation occurs at a ratio of 1.41×10^{-2}M TEPP/M protein, or a calculated uptake for complete inhibition (in view of the linear concentration-inhibition curve [*2*] of 2.82×10^{-2}M TEPP/M protein). This figure is in fair agreement with that calculated above if correction is made for C^{14} uptake that does not appear related to the enzyme-inhibitor reaction.

The data presented indicate the formation of a stable compound between plasma cholinesterase and a small fraction of the hexaethyl tetrapolyphosphate required to inhibit the enzyme. From 82 to 88% of the C^{14} fixation thus obtained appears to be related to the enzyme inhibition, the rest being accounted for by a much less specific reaction. Calculations have been presented indicating that the amount of C^{14} taken up in connection with the specific reaction is close to the amount of tetraethyl pyrophosphate required for complete inhibition of the enzyme, again suggesting that the anticholinesterase activity may reside in this or closely similar compounds of this group. Finally, an estimate of the molecular relations between HETP and the enzyme has been presented; a reasonable value (about 3%) for the purity of the enzyme preparation employed could be arrived at on the assumption of a mean molecular weight of 3×10^5 for the protein, and of a reaction involving 1 mol of HETP and 1 mol of protein.

References

1. Ammon, R. *Pflugers Arch. f.d. ges. Physiol.*, 1934, **233**, 486.
2. Brauer, R. W. *J. Pharm. exp. Therap.*, 1948, **92**, 162.

Iron Metabolism.[1] Heme Synthesis *in Vitro* by Immature Erythrocytes

R. J. Walsh, E. D. Thomas, S. K. Chow, R. G. Fluharty, and C. A. Finch

Department of Medicine, Harvard Medical School, The Medical Clinic, Peter Bent Brigham Hospital, Boston, and Radioactivity Center of the Laboratory of Nuclear Science and Engineering, Massachusetts Institute of Technology, Cambridge

Studies with radioiron have indicated that there is no exchange of iron between the mature erythrocyte and surrounding plasma (*3*, *4*). The following data, however, indicate that reticulocytes will assimilate iron and synthesize heme *in vitro*, and that this uptake of radioiron may be used as an indicator of the rate of hemoglobin synthesis.

Fe^{59} and Fe^{55} with specific activity of about 20 and 200 μc/mg iron respectively were prepared at the Massachusetts Institute of Technology cyclotron. Blood with an increased content of reticulocytes from patients with pernicious anemia during a response to liver and from patients with iron deficiency, and bone marrow from rats and humans were used. *In vitro* studies were performed in rocking boats at 37° C in a gas mixture of 95% oxygen and 5% CO_2, as previously described by Geiman and his associates (*2*), over a period from 4 to 24 hr. Those experiments extending beyond 6 hr were carried out with sterile precautions.

[1] Assisted by the joint program of the Office of Naval Research, the Atomic Energy Commission, by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service and, in part, through support from Smith, Kline, and French.

While blood containing less than 1% reticulocytes took up no measureable quantity of radioiron, uptake of radioactivity was demonstrated repeatedly in blood with a high reticulocyte content. That the radioactivity was localized in the immature cell population was shown by correlation of the reticulocyte count and radioactivity

TABLE 1

	Reticulocytes	Cpm/ml red cells
Top (low cell specific gravity)	53%	500
Bottom (high cell specific gravity)	5%	35

in various fractions of this blood separated by the albumin flotation technique (*7*).

Table 1 shows such a separation of whole blood previously incubated for 4 hr with radioiron, washed five times in ten times its volume of 1% saline, and then partitioned by albumin flotation.

Various types of blood were studied, including iron-deficiency anemia, acquired hemolytic anemia, sickle cell anemia, and pernicious anemia. The uptake of iron in all instances was attributable to the presence of reticulocytes. It would further appear that in pernicious anemia at least the rate of uptake is also related to the type of reticulocyte present. The early reticulocytes after liver therapy contain more reticular material and pick up more radioactivity, as shown in Fig. 1.

Conditions necessary for the uptake of radioiron have been studied to a limited degree. Ferrous iron added to a saline suspension of erythrocytes containing an increased number of reticulocytes is taken up perhaps twice as rapidly as ferric iron. Iron carried by the iron-binding protein of the serum (*5*) is assimilated by the reticulocyte but less rapidly than the inorganic ferrous and ferric iron. The iron uptake is impaired by lack of glucose in the media and by low temperature. In an effort to determine whether the uptake of iron indicated hemoglobin formation, red cells have been fractionated after incubation with radioiron. The largest amount of activity in the reticulocyte portion was found in the stroma of the hemolyzed cells. However, significant amounts of radioiron were also demonstrated in recrystallized heme from these cells. These observations indicate that the physiological process of the assimilation of iron by the developing red cell is, first, the attachment of iron to acceptors in the red cell stroma capable of removing iron from the serum and second, the synthesis of heme. These studies also indicate that reticulocytes are still capable of completing the process of hemoglobinization in the peripheral blood, in keeping with observations that tagged reticulocytes live a normal life span (*1*).

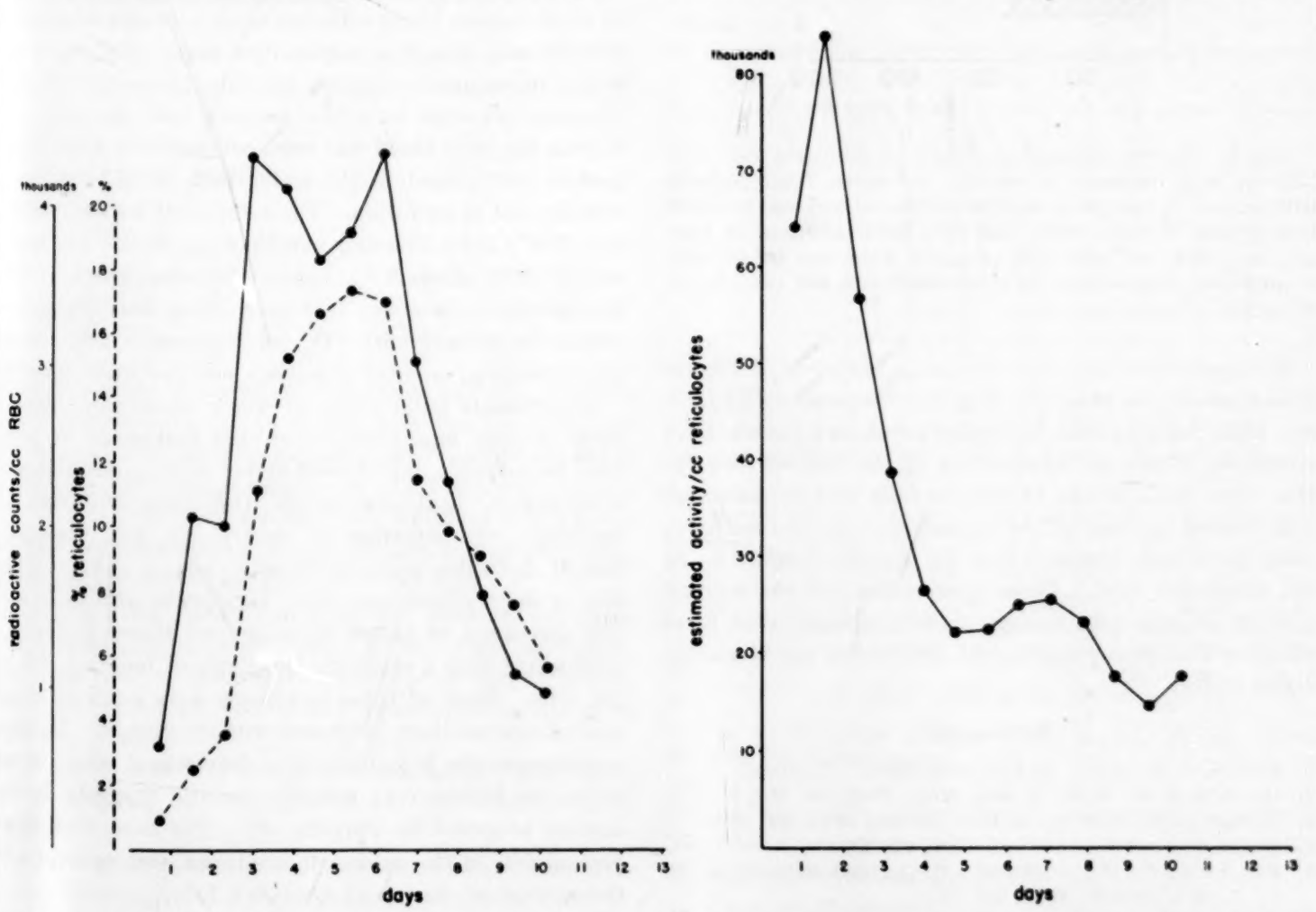

FIG. 1. Incubation of blood with radioiron *in vitro*. The patient studied had pernicious anemia and was given 60 units of liver concentrate on 0 day. On each subsequent day 20 ml of blood was drawn and incubated with radioiron over a period of 6 hr. The % reticulocytes and uptake of radioiron per ml of blood and per ml reticulocytes are shown above. It is assumed in the calculation of activity per ml reticulocytes that only reticulocytes take up radioiron.

The dotted line in the figure indicates % reticulocytes; the solid line indicates radioactive counts.

Similar incubation studies have been conducted on bone marrow aspirations. As might be expected from the spectrophotometric studies of Thorell relating to hemoglobin synthesis (*6*), the uptake of radioiron by normoblasts is much greater than that observed in reticulocytes. Again, radioactive heme could be demonstrated after incubation of radioiron with these marrow cells.

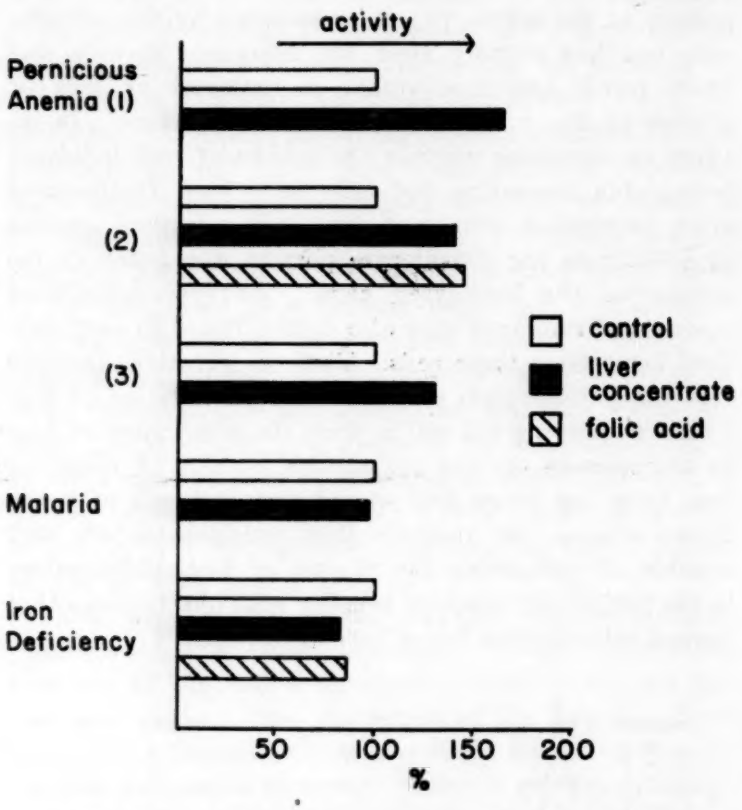

FIG. 2. Marrow incubation. Effect of folic acid and liver. Marrow cells obtained by sternal aspiration from patients with untreated pernicious anemia showed an increase in radioiron uptake of 66%, 44%, and 28% after addition of liver (1×10^{-5} unit) or folic acid (5 μg). This was in contrast to slight decreases found in other conditions not involving a deficiency in these substances.

It is possible to use radioiron as an indicator of altered hematopoiesis, as shown in Fig. 2. Suspensions of marrow from patients with untreated pernicious anemia have repeatedly shown an acceleration of the rate of iron uptake after the addition of liver or folic acid as compared with control studies. This has not occurred in conditions other than those characterized by specific deficiencies of the substances used. These observations on the marrow cells in untreated pernicious anemia indicate that these effective therapeutic agents act directly on the immature erythrocytes.

References

1. FINCH, C. A. *et al.* *J. Lab. Clin. Med.* In press.
2. GEIMAN, Q. M. *et al.* *J. exp. Med.*, 1946, **84**, 583.
3. GIBSON, J. G. II *et al.* *J. clin. Invest.*, 1946, **25**, 616.
4. HAHN, P. F. *et al.* *Science*, 1940, **92**, 131.
5. SURGENOR, D. M., KOECHLIN, B. A., and STRONG, L. E. *J. clin. Invest.*, 1949, **28**, 73.
6. THORELL, B. *Studies on the formation of cellular substances during blood cell production.* London: Henry Kimpton, 1937–1947.
7. VALLEE, B. L., HUGHES, W. L., and GIBSON, J. G. II. *Blood*, 1947, Special Issue No. 1, 82.

Freezing of Whole Blood

Max M. Strumia[1]

John S. Sharpe Research Laboratory
and the Laboratory of Bryn Mawr Hospital,
Bryn Mawr, Pennsylvania

It is a generally accepted hypothesis that freezing and thawing of red cells results in hemolysis. Alternate freezing and thawing is a procedure commonly employed in the laboratory for the purpose of obtaining hemolysis. Preliminary experiments carried out in 1942 showed that the hypothesis is not correct under all conditions, i.e., it is possible to freeze whole blood in a solid mass and thaw it without appreciable hemolysis. "Freezing of blood" is here intended to mean solidification of blood by means of temperatures well below the freezing point of blood.

Early in our experiments, evidence showed that the type of water crystallization resulting from freezing was not an essential factor in the behavior of the red cells. Thus, equally good preservation of red cells was obtained by slow freezing at −3° C or by very rapid freezing at −60° C. In the case of slow freezing, the mass of frozen blood showed formation of large crystals; whereas with fast freezing, the mass of frozen blood appeared very uniform. Fast freezing was carried out as follows: 1 ml of whole human blood collected in an acid-citrate-dextrose mixture was placed in a glass test tube. The blood was frozen by manually rotating the tube in cracked CO_2 ice. Freezing occurred in a few seconds, and the tube containing the solid blood was removed instantly upon solidification and placed in the water bath at 37° C to thaw with the aid of agitation. The hematocrit before freezing was 39.37; after freezing and thawing, 39.07; the supernatant fluid showed no appreciable discoloration from hemoglobin. However, if frozen blood was allowed to remain in contact with CO_2 ice for even a few seconds after freezing, massive hemolysis resulted upon thawing.

Experiments on freezing of whole blood were resumed about a year ago. More than 150 specimens of blood have been frozen and thawed under varying conditions of temperature, heat dissipation (affecting the time of freezing), concentration of electrolytes, pH, concentration of diffusible and nondiffusible sugars (affecting the size of the erythrocytes), etc. In a series of a little over 100 specimens of blood, freezing and thawing were accomplished with a resulting hemolysis of less than 1% of the cells. Most of these specimens were collected in an acid citrate solution, with and without glucose. In these experiments the hematocrit was determined with the use of an air turbine (*2*) and the osmotic fragility by the method proposed by Parpart (*3*). The concentration of hemoglobin in the supernatant plasma was measured by the method of Karr and Chornock (*1*).

It is of practical importance to note that even when freezing and thawing of red cells at −3° C results in

[1] With the technical assistance of Miss Margaret M. Dolan and Miss Louise Colwell.

some hemolysis, the remaining red cells appear to be undamaged.

Experiments have shown that regardless of the mode of freezing of blood, rapid thawing at +37° C in the water bath with agitation is the best method to avoid hemolysis. Results similar to those reported for whole citrated blood may be expected, and have been obtained, with red cells suspended in various media. Much better results have been obtained when red cells are frozen after crenation produced by a hypertonic solution of sucrose, than when red cells are swollen by the addition of a hypotonic solution of glucose.

The preservation of blood in the frozen state is of particular interest for the obvious practical advantages which it offers. In all, about 100 specimens of blood have been satisfactorily preserved for varying periods of time up to 1 month.

The importance of temperature control for proper preservation of blood in the frozen state is best emphasized by the following experiment: 500 ml of whole blood were mixed with 75 ml of chilled anticoagulant solution.[2] Blood was maintained at room temperature for the first 3 hr. It was then distributed in 10-ml aliquots in rubber-stoppered, thin-walled test tubes. A tube (#8) was placed in a rapidly circulating water-alcohol mixture cooled to −14° C. The blood in the tube was agitated and the thermometric readings were as follows:

Time (min)	Temperature (° C)
0	+21
1	+ 6
2	+ 1
3	± 0
4	− 1
5	− 2.4
6	− 0.455

Initial freezing occurred at this point, and the tube was allowed to remain in the cooled bath for an additional 14 sec. While the thermometer still registered about −0.455° C, the tube of semifrozen blood was removed and placed at −3° C in an air cabinet. Within a few minutes at this temperature the blood became completely solid, and was maintained solid at −3° C for 1 hr.

Tube #3 was treated in a similar manner, but after initial freezing at about −0.5° C, the tube was maintained at −14° C for a little over 3 min; this tube then was also placed at −3° C and maintained in the solid state for 1 hr.

A control tube (C) contained whole blood preserved in A.C.D. solution and maintained at +4° C for about 4 days.

Results of hematocrit determinations, and of determinations of hemoglobin in the supernatant fluid, and of osmotic fragility in hypotonic salt solution after thawing are shown in the table and figure.

The results may be summarized as follows: When whole citrated blood is frozen to a solid mass in a cooled circulation bath, removed as soon as freezing has started and placed at −3° C (+0.5) for periods of several hours, minimal hemolysis and minimal changes in the hematocrit and osmotic resistance are noted comparable to that obtained with whole blood preserved in A.C.D. solution at +4° C for 4 days. When frozen blood is allowed to cool for even a very short period of time at temperatures below −3° C, rapid and severe hemolysis occurs.

When whole citrated blood is placed in an air cabinet, cooled at −3° C and allowed to remain undisturbed, it will remain liquid for an indefinite period of time. This has allowed comparative study of blood preserved at −3° C in the solid and in the liquid states.

TABLE 1

HEMATOCRIT READING AND HEMOGLOBIN OF SUPERNATANT PLASMA OF WHOLE BLOOD FROZEN AND PRESERVED FOR 1 HR AT −3° C IN THE FROZEN STATE

	Hematocrit readings	Hemoglobin in supernatant plasma
Control	36.61	3 mg %
Tube #8*	36.84	6 mg %
Tube #3†	30.40	240 mg %

* Tube #8 was properly frozen and thawed.

† Tube #3 was allowed to remain too long after initiation of freezing in the bath at −14° C. The discrepancy between the hemoglobin in the supernatant plasma and the hematocrit reading is due to the fact that high centrifugal force, developed in the air turbine used for hematocrit determinations, breaks damaged red cells. This discrepancy is always an indication of poor preservation of red cells. The hematocrit of damaged cells is generally difficult to determine and not too reliable.

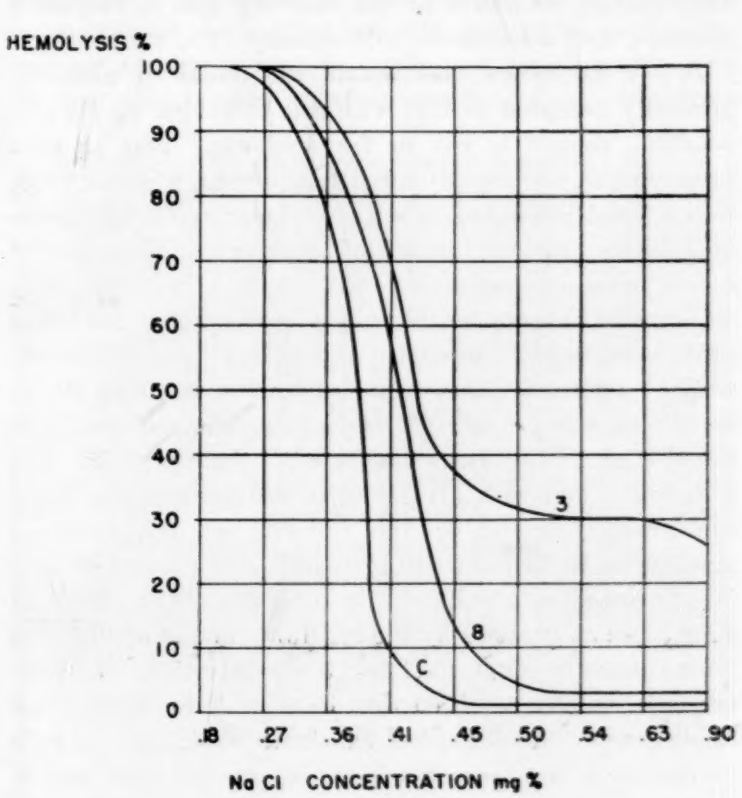

FIG. 1. Fragility of frozen whole blood stored for 1 hr at 3° C in the frozen state.

[2] Standard A.C.D.

The results obtained so far in the preservation of frozen and liquid blood at −3° C are sufficiently encouraging to justify further studies, which are now under way.

References

1. KARR, W. G. and CHORNOCK, F. W. *J. clin. Invest.*, 1947, **26**, 685.
2. PARPART, A. K. and BALLENTINE, R. *Science*, 1943, **98**, 545.
3. PARPART, A. K. *et al.* *J. clin. Invest.*, 1947, **26**, 636.

A "Free Manometer" Method of Using the Standard Warburg Apparatus[1]

Avram Goldstein

Department of Pharmacology, Harvard Medical School, Boston,* and *the Marine Biological Laboratory, Woods Hole, Massachusetts

In the Warburg apparatus, as commonly employed, a gas reaction occurs within a closed system of constant volume. The temperature being held constant, pressure changes (read on the open manometer arm) are related by a linear function to the volume of gas evolved or taken up. This function is known as the "vessel constant" and depends upon gas-space volume, fluid volume, temperature, solubility of the reacting gas, atmospheric pressure, and manometer fluid density (*1*).

It can be shown that within the limits of accuracy generally accepted in the Warburg technique (±1%), a constant volume is not in fact required; that if both pressure and volume are permitted to vary, a linear function (vessel constant) is still obtained; and that consequently the repeated leveling of manometer fluid, whereby a constant volume is maintained, can be eliminated.

The free manometer technique presents the following unique features: automatic recording, by a time-controlled camera, becomes feasible; certain small errors inherent in the process of leveling the manometer fluid are eliminated; readings can be made more rapidly, more frequently, and with greater ease, and arithmetical steps are greatly simplified; the capacity of the standard manometer is substantially increased, while its sensitivity is correspondingly reduced. A total gas change three to four times as great as by the constant volume method can be measured without resetting the manometer. This increased capacity has been found desirable in at least two applications: in measuring substrate oxidation by cells or tissues in the face of a high endogenous rate, and in studying the protracted time course of enzyme-substrate reactions.

The fluid on the vessel side of the manometer is set initially to the manometer midpoint and subsequent readings are made on this same arm of the manometer. The fluid adjustment is not touched again after the initial setting. A reciprocal and equal change occurs in the fluid levels of the two arms as a reaction proceeds, but the fluid in the open arm is ignored. Thermobarometer corrections, provided they are not exceedingly large (< 5 mm), are made in the usual fashion, but the thermobarometer vessel must contain the same volume of fluid as the other vessels.

If the vessel constants are determined empirically no special problems arise. If they are calculated from mercury calibration of the gas space an additional factor M is required. M is the linear volume of the manometer (cu mm/mm) and is obtained automatically if one follows the calibration method suggested by Burris (*3*, p. 50), filling the manometer first to a point above, and then to a point below the midpoint mark. The full vessel constant equation is:[2]

$$k = \left[\frac{V P_o + V_f RT\alpha_x}{P_o RT}\right]\left[\frac{p_x P_o M}{V P_o + V_f RT\alpha_x} + \frac{p_z P_o M}{V P_o + V_f RT\alpha_z} + 2\right]$$

It should be noted that the only variables requiring calibration are V and M. The observed change in level (mm) on one arm of the manometer multiplied by the vessel constant gives moles of gas reacting at NTP.

The full equation must be used for CO_2, but O_2 and other gases of low solubility can be determined with the following simplified vessel constant:

$$k = \frac{2V + M\,(P_o - p_w)}{RT} + V_f\,\alpha_x\left[\frac{2V + M\,(P_o - p_w)}{V P_o}\right]$$

where p_w is the vapor pressure of water (mm manometer fluid) at temperature T.

The method described here has been in use in this laboratory for some time. Replicate determinations of CO_2 and O_2 by free manometer and constant volume techniques under diverse conditions agree satisfactorily. It has proved convenient to calculate vessel constants by both methods and to use the free manometer technique routinely, reserving the constant volume technique for those occasions when high sensitivity is desired. Full theoretical and practical details of the method will be published elsewhere (*2*).

References

1. DIXON, M. *Manometric methods, as applied to the measurement of cell respiration and other processes.* 2nd Ed. New York: Macmillan, 1943.
2. GOLDSTEIN, A. *Proc. Amer. Acad. Arts Sci.*, 1949, 77, No. 7.
3. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. *Manometric techniques and related methods for the study of tissue metabolism.* Minneapolis: Burgess, 1945.

[1] The work reported in this paper was done during tenure of a Lalor Fellowship and was also supported by a grant from the Abbott Laboratories, North Chicago, Illinois.

[2] V = gas space (cu mm)
M = manometer factor (cu mm/mm)
$R = 8.21 \times 10^4 \times P_o$
T = absolute temperature
p_x = initial partial pressure of reacting gas (mm manometer fluid)
p_z = initial partial pressure of inert gas (mm manometer fluid)
P_o = atmospheric pressure (mm manometer fluid)
V_f = fluid volume (cu mm)
a_x, a_z = solubility coefficients of the gases (moles/cu mm at P_o, T)

A Method of Optically Recording Contractions and Electrocardiograms from Isolated Frog Hearts

F. D. McCrea and Sydney Ellis

The Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina

This method was devised for recording simultaneously the muscular contractions and electrocardiograms from the isolated frog heart. The apparatus consists of a glass tube, 15-mm bore and 6–7 cm long, with two short side arms about 1 cm from the top and bottom, respectively, of the tube. The bottom is sealed by a rubber stopper through which projects a small glass tube containing an electrode projecting into the tube and which is vertically adjustable within the tube. The lower side arm is connected by rubber tubing with a small leveling bulb, which is filled half-full with the control solution used for perfusion (e.g., Ringer's or Clark's). This

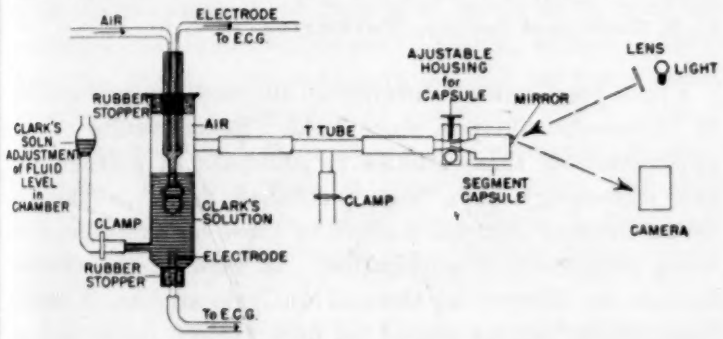

FIG. 1. Diagram of apparatus.

bulb is convenient for adjusting the fluid level in the tube, which is filled about half-full with the solution.

The heart is perfused by the method of Straub. The perfusion cannula bearing the frog heart passes through a rubber stopper of proper size to close the top of the tube. The heart is then put in the tube, and the cannula adjusted so that the heart is just immersed in the control solution. The tube thus becomes a modified cardiometer. The upper side arm is connected by a short piece of 2-mm bore rubber tubing bearing a T tube to equalize pressure, with a Wiggers (*1*) segment capsule. The capsule is covered by a moderately stretched condom diaphragm which carries the mirror. The second electrocardiographic electrode consists of a German silver wire about 18 gage which is passed down inside the perfusion cannula so that its tip is just above the constriction of the cannula. The perfusing solution, and the control solution in which the heart is immersed, adequately serve as conductors. A diagram of the apparatus appears in Fig. 1.

A variant of this procedure consists in attaching the tip of the ventricle by means of a clip and fine wire conductor to a small flat tambour loosely covered by a condom diaphragm. The wire is attached to the center of the tambour diaphragm so that a very slight tension is maintained on the heart. One electrode is then connected with this wire. The tambour outlet is then connected with the segment capsule as above. No solution surrounds the heart in this case. This method was found to be inadequate to record feeble contractions.

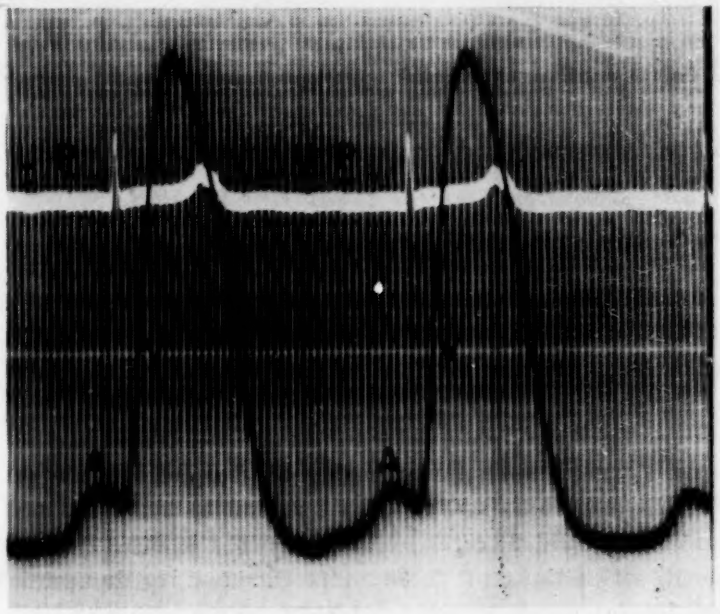

FIG. 2. Electrocardiogram and cardiometer curves. *U* and *P* waves show as small deflections. *A* and *V* indicate auricular and ventricular volume changes.

The cardiometer in our experience has proved to be adequate to record any visible contraction. It affords much greater flexibility, and the sensitivity of the recording apparatus may be easily and rapidly altered by varying the volume of air above the fluid. We have found some 5 cm^3 of air in the tube a convenient volume to secure regularly an excursion of 8–10 cm from normal frog hearts.

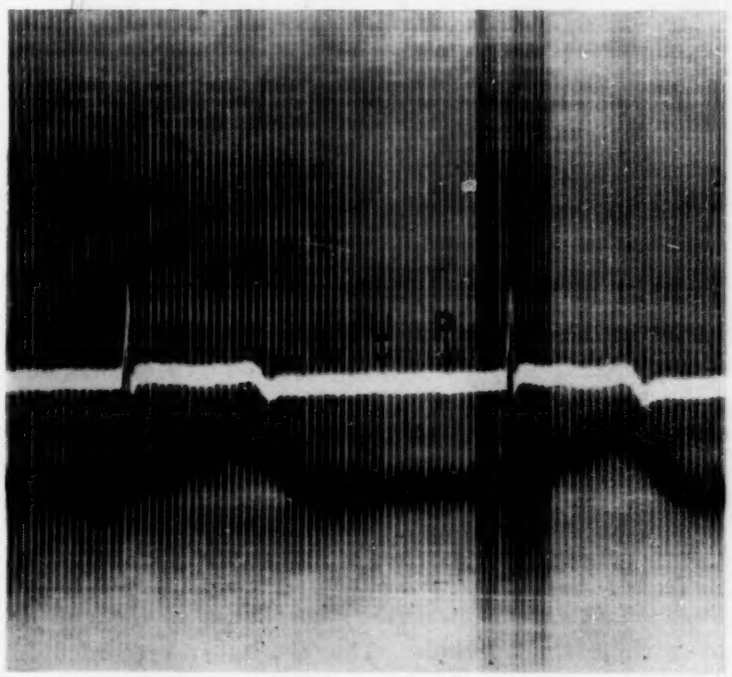

FIG. 3. Above—Electrocardiogram. Below—ventricular myogram.

Fig. 2 was obtained using the first method described. Fig. 3 was obtained by the second method.

Reference

1. WIGGERS, CARL J. *J.A.M.A.*, 1915, **64**, 1305.

Hydrolysis of Adenosine Triphosphate by Trichloracetic Acid[1]

Adolph Bernhard and Louis Rosenbloom

Achelis Laboratories of the Lenox Hill Hospital, New York City

In the determination of adenosine triphosphatase activity, the usual procedure utilizes addition of trichloracetic acid for precipitation of the protein and stopping of the enzyme action. The estimation of inorganic phosphorus is then done on the filtrate. In determining dephosphorylation of an adenosine triphosphate substrate by human serum (*2*), we had occasion to leave the trichloracetic acid filtrates overnight in the icebox. Subsequent estimations of these same filtrates for inorganic phosphorus revealed values which were much higher than those previously obtained. Our interest in this casual observation led us to undertake an investigation of this phenomenon.

Experimentally, a solution of sodium adenosine triphosphate[2] in veronal-HCl buffer at pH 8.9, in a concentration of 1 mg in 1 ml of buffer was used. Filtrates were prepared in the following manner: to each 2.5-ml portion of the buffered Na-ATP solution 0.5 ml of water (instead of serum) and 5 ml of 8% trichloracetic acid was added, and the resulting solution was well mixed and then filtered. The inorganic phosphorus was determined on 4 ml of the filtrate by the method of Fiske and Subbarow (*1*). Sufficient filtrate was made to insure duplicate estimations of inorganic phosphorus at the end of the various time periods. The results are recorded in Table 1.

TABLE 1

HYDROLYSIS OF NA-ATP BY TRICHLORACETIC ACID

Sample	Preparation	Control	Time in hr		
			2	4	24
		μg P/ml			
1	Na-ATP 1 mg in 1 ml of veronal-HC1 buffer, pH 8.9, room temperature	4.1	6.3	8.1	27.3
2	Do., in icebox	4.1	5.1	5.8	10.3
3	Na-ATP 1 mg in 1 ml H_2O, room temperature	4.4	5.5	7.9	
4	Do.	2.4	6.8	8.4	

In order to obviate the possible effect of the buffer solution employed, an aqueous solution of Na-ATP in the same concentration was prepared and treated in the same manner as described above. These results are also shown in Table 1.

It is evident that there is a definite increase in inorganic phosphorus when adenosine triphosphate in trichloracetic acid is allowed to stand at either room or icebox temperature. No such change takes place when solutions of adenosine triphosphate without addition of trichloracetic acid are allowed to remain for the same periods of time. Trichloracetic acid filtrates prepared from serum alone do not hydrolyze under similar conditions of time and temperature.

The results indicate that spontaneous hydrolysis of adenosine triphosphate by trichloracetic acid does occur. It is essential that determinations of inorganic phosphorus be made under identical conditions in order to avoid errors due to such hydrolysis.

[1] Aided by a grant from the Frederick Kolb Fund.

[2] The tetra sodium salt of adenosine triphosphate · $3H_2O$ was obtained from Rohm & Haas, Philadelphia, Pennsylvania.

References

1. FISKE, C. H. and SUBBAROW, Y. *J. biol. Chem.*, 1925, **66**, 375.
2. MEISTER, A. *J. clin. Invest.*, 1948, **27**, 263

Differentiation of Aragonite from Calcite by Differential Thermal Analysis

George T. Faust

U. S. Geological Survey, Washington, D. C.[1]

I have been making differential thermal analysis studies of carbonate minerals since 1942. Some results of the application of these studies to problems in petrography and mineralogy have been published (*2*, *4*). The detailed thermal analysis studies on these minerals are now being prepared for publication. In view of the current interest in differential thermal analysis studies, it seems desirable to put on record an observation, made several years ago, on the identification of aragonite by this method.

The differential thermal analysis apparatus used in these studies is almost identical with the one designed by Hendricks, Alexander, and Nelson (*1*, *5*), and with a sensitivity dependent on resistance in series with the galvanometer of 999.9 ohms.

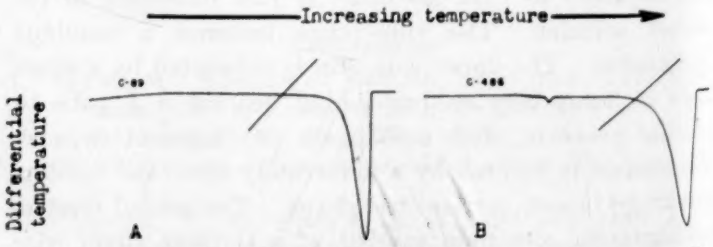

FIG. 1. (A) Calcite from Joplin, Missouri. (B) Aragonite from Chile.

Typical curves for aragonite and calcite are given in Fig. 1. The short diagonal lines are portions of the curve used to determine the temperature of the thermal reactions recorded (*3*). The principal curve shows the course of the thermal reactions.

The calcite sample, illustrated in Fig. 1A, is from Joplin, Missouri, and is part of the tube sample analyzed by R. C. Wells (*6*). The curve shows one large peak representing the dissociation of calcium carbonate into

[1] Published by permission of the Director, U. S. Geological Survey, Washington, D. C.

calcium oxide and carbon dioxide. The peak temperature for this sample is 972° C. The aragonite (U.S.N.M.-R2554) is from Chile, exact locality unknown, and its thermal curve is shown in Fig. 1B. This curve shows a small peak at 447° C which represents the dimorphic transformation of aragonite to calcite. This transformation of a metastable material is irreversible, and hence does not take place at a reproducible temperature. Subsequently, the calcite, paramorphous after aragonite, undergoes decomposition at 897° C. The temperature of the dissociation of calcite is not a definite temperature in a nonequilibrium process. The presence of the low temperature peak, in this sample of aragonite at 447° C, representing the transformation of aragonite to calcite, serves to differentiate these two minerals. However, this peak requires a sensitive, continuous recording apparatus and can easily be overlooked.

References

1. Alexander, L. T., Hendricks, S. B., and Nelson, R. A. *Soil Sci.*, 1939, **48**, 273, Part II.
2. Faust, G. T. *Econ. Geol.*, 1944, **39**, 142.
3. ———. *Amer. Mineralogist*, 1948, **33**, 337.
4. Faust, G. T. and Callaghan, E. *Geol. Soc. Amer. Bull.*, 1948, **59**, 11.
5. Hendricks, S. B. and Alexander, L. T. *Soil Sci.*, 1939, **48**, 257, Part I.
6. Wells, R. C. *J. Wash. Acad. Sci.*, 1915, **5**, 618.

Comments and Communications

The Preparation of Graphs for Publication

In the preparation of graphs of many sorts, it is exceedingly convenient to use graph paper. Graphs so prepared are satisfactory for study but not for publication, because of the background of fine lines. If blue-lined paper has been used, these lines can be eliminated by photography; but this requires some experience and skill and, for some of us, is apt to mean more than one attempt. Moreover, blue-lined paper is not always available.

Recently I noticed that the lines on graph paper show through the paper well enough for the fixing of points on the back. The graph paper is placed upside down on a white surface, and the portion of the sheet to be used is outlined and certain reference points are indicated before the data points are marked. The finished graph, in ink on the plain white back of the paper, may be submitted directly or conveniently photographed.

This method may be helpful to many investigators where professional draftsmen are not available.

James S. Gutsell

U. S. Department of the Interior,
Fish and Wildlife Service
Kearneysville, West Virginia

Use of Omental Spread in Biological and Pathological Studies

The advantages of using the omentum of a small animal for making spread preparations instead of attempting to section the tissue have been pointed out by Cross (*Science*, 1949, **109**, 314). However, the technique described, of first taking up the desired portion of omentum on a bit of cigarette paper and then transferring it to a slide, is needlessly complicated. Instead, a piece of omentum may be removed and placed directly on a slide (Woodruff, C. E. *Amer. J. Path.*, 1934, **10**, 739). Then, making use of the property of this thin membrane of fixing itself to glass, one corner of the omentum may be teased out and allowed to dry on the slide. From this anchoring corner the balance of the membrane may be teased out to form a thin layer most of which will be only one cell thick. Certain stains such as the Ziehl-Neelson may be used on the omental spread without further fixation. Other stains may require the use of formalin or some other fixative prior to staining. The stained preparation is readily mounted in balsam and affords a three-dimensional picture of developing disease processes which one cannot obtain by the use of conventional histological sections.

Very satisfactory omental spreads may be obtained from mice and guinea pigs. The omenta of rabbits and dogs are of less value for this purpose, since the tissue fails to become fixed to the slide in a satisfactory manner.

C. Eugene Woodruff

William H. Maybury Sanatorium,
Northville, Michigan

Calibration of Warburg Manometers

The paper by Loomis (*Science*, 1949, **109**, 491) concerning the calibration of Warburg manometers omits mention of the convenient method of Schales (*Arch. Biochem.*, 1944, **3**, 475), which also involves the principle of filling the manometers from below by means of a mercury reservoir. Schales' method possesses the further advantage of not requiring any spatial manipulation of the manometers in order to adjust the level of the mercury.

The disadvantage of Schales' method, in our experience, has been the difficulty of placing just the right amount of mercury in the flask in order that the fluid will rise to the point of junction of the side arm and the manometer when the flask is slipped on the ground joint. We have avoided this difficulty by placing a minimum amount of mercury in the flask (usually to the lower edge of the ground surface), and adding further amounts through the gas outlet tube with a capillary

pipette after the flask is attached to the manometer. The gas outlet tube is closed to the flask as it is filled with mercury; a small pressure bulb is attached to the tube, and it is rotated so as to be open to the flask. The mercury is gently blown into the flask, thus filling the side arm. This operation is repeated as often as necessary and takes only a few seconds.

This refinement of Schales' procedure eliminates the necessity for spilling even a drop of mercury.

R. E. MAXWELL

Iowa State College and Iowa Agricultural Experiment Station, Ames, Iowa

Correction

Referring to my paper "Microcrystallographic Data on Sodium-D-Glutamate (Monosodium Glutamate)", *Science*, 1949, **110**, 304, Dr. B. F. Buchanan, International Minerals & Chemical Corporation, Chicago, informs me that the monosodium glutamate upon which this study was made is the L-form and should be designated as sodium-L-glutamate, being the monohydrate with the following empirical formula: $C_5H_8O_4NNa \cdot H_2O$.

GEORGE L. KEENAN

Strongsville, Ohio

Oral Stress and Meaning in Printed Material

In experiments conducted by the writers, evidence has been secured indicating that the ability of the subject to understand a prose passage may be given in quantitative terms by noting which words the subject stresses as important when he reads the passage aloud.

This apparently novel observation, so far as measurement is concerned, reinforces a belief which many teachers of reading hold, that certain assets have been sacrificed in the current emphasis on silent reading as contrasted with the older oral reading methods. Our conclusion was developed in relation to experiments on the electrical recording of eye movements during long-continued reading of prose set in type forms arranged to emphasize certain ideas.

In the printed material so far studied, words which should be stressed are defined as those words which the author or competent judges stress when they read the material aloud. It has been discovered that readers who stress words which the author indicates should be stressed obtain significantly superior scores when given a written comprehension test on the same reading material. The association between a subject's ability to differentiate between delicate levels of stress and his comprehension of the material read is marked. Correlation coefficients ranging from 0.45 to 0.65 have been consistently found in several hundred high school and college subjects so far studied.

It is not necessary for subjects to read aloud to demonstrate the association between stress and meaning. It is enough that subjects be instructed to mark those words that they would stress were they reading the material in question aloud. The determination of which words the subject would stress can be made conveniently by presenting the subject with a multiple-choice test.

That the *manner* in which a passage is read is correlated substantially with understanding of the passage has implications with respect to methods of teaching reading at the primary grade levels. This finding suggests that more emphasis on oral reading than has recently been recommended may be appropriate.

This relationship between oral stress and meaning is important in considering the possibility of improving the efficiency of print as a transmission system. Attempts have been made down through the years to introduce boldfaced type or italics and to spread out letters as means of indicating stress. Aesthetic and other arguments have been used by those who oppose the use of such varied type. It is clear, however, that vocal stress supplies the listener with information over and above the information which he would receive if speech were conducted exclusively in monotones and at an unchanging rate. It appears a matter for regret that this added oral meaning has not yet been introduced into print in a form which is acceptable. The results of the present experiment suggest that the opposition to the use of varied type has less weight now that there is available a working principle such as is here presented for indicating *consistently* which words or phrases should be stressed.

Our study so far indicates that when a reader wishes to glean the last bit of meaning from a written document he prefers stress indications in the printed matter if he is offered a choice between stressed material and the conventional unstressed material. As an illustration, a group of students who were to be examined in part upon the lectures previously given by a college instructor were offered a choice between a set of notes in standard type or a set of notes arranged so that the print showed the actual vocal stresses which the instructor employed when delivering the lectures. A majority of students preferred the notes printed so as to indicate oral stress. There are other situations where exact understanding is of such critical importance that readers prefer stresses to be shown in the printed documents. Some military orders and directives, for example, fall in this category.

The discovery of the relatively high correlation between the ability to understand and the ability to indicate stressed words, as outlined in this note, is being used in further experiments by the authors on changing the readability of prose. It is anticipated that this less ambiguous system for the transmission of meaning in print will result in decreasing significantly the number and duration of fixation pauses and movements of the eye which are ordinarily required in reading. These experiments are being conducted with due consideration for the aesthetics of the printed page and for modern typographic conventions.

W. F. DEARBORN, P. W. JOHNSTON, and L. CARMICHAEL

Tufts College, Medford, Massachusetts

Book Reviews

Solid analytic geometry. Adrian Albert. New York: McGraw-Hill, 1949. Pp. ix + 162. $3.00.

The first five chapters of this textbook present the usual material on lines, planes, spheres, and the other quadric surfaces, concluding with an invariantive classification of quadrics. The concepts and terminology of vector algebra are introduced immediately and are used effectively throughout. The sixth chapter is devoted to the theory of matrices, which in the seventh chaper is applied to rotations in space and to the principal axis transformation. The last two chapters are considered by the author to be supplementary to a basic course. Chapter 8 is on spherical coordinates and gnomonic charts, useful material which is frequently neglected. Chapter 9 is an introduction to analytic projective geometry in the plane and in space, carried far enough to include a proof by means of matrices of the invariance of the cross ratio.

The treatment throughout is designed to tie analytic geometry to the modern theory of vectors and matrices, and the methods employed are for the most part those which can be generalized directly to the Euclidean geometry of n dimensions. The book contains much excellent material illustrating the applications of the theory of vectors and matrices to solid Euclidean geometry and many short and elegant proofs are developed in this technique. The only criticism which occurs to the reviewer is that the viewpoint is predominantly algebraic rather than geometric, and that there is a minimum of emphasis on developing the student's space intuitions.

C. C. MacDuffee

University of Wisconsin

Lehrbuch der Embryologie. Walter Brandt. Basle, Switzerland: S. Karger, 1949. Pp. xii + 648. (Illustrated.) 56 Swiss francs.

This is a textbook of human embryology for medical students. In general, it follows the standard pattern: a rather extensive chapter on general embryology is followed by chapters on organogenesis. The text is written clearly and concisely, and the important chapters on human placentation, fetal circulation, and hormonal control of the sexual cycles, in which fields much recent work has been done, are up to date. The illustrations, partly in color, are adequate for the most part but do not do justice to the chapters on the nervous system and the sense organs. For instance, illustrations of the histogenesis of the central nervous system and of the structural differentiation of the eye and ear and their auxiliary structures are entirely missing. Moreover, illustrations of such important processes as fertilization and gametogenesis (which latter is represented by a poor diagram) would have been desirable.

An innovation of the book is the inclusion of a considerable amount of experimental embryological material. This reviewer is wholeheartedly in favor of a more analytical and dynamic approach to embryology than is offered by most texts. It is, therefore, regrettable that these parts do not live up to expectation. The promise of the author, in the foreword, to incorporate the newest results in developmental physiology is not kept in the text which, apart from very few exceptions, considers the literature only up to 1934. As a result, the presentation of the subject and the viewpoints are outdated and inadequate in many respects. For instance, our conceptions of embryonic induction and of axis determination in limb primordia have undergone considerable changes in the last fifteen years. The old ghosts of mosaic- and regulation-eggs are revived, and the descriptions of gastrulation in Amphibia and in higher forms, and of the fundamental processes of the segregation of organ primordia from the germ layers, are incorrect. In the part on organogenesis, the experimental data on the determination of an organ are presented in advance of the description of its embryonic development; the didactic wisdom of this procedure is questionable. A serious shortcoming of the book is the lack of a bibliography, in spite of numerous bibliographic references in the text. It is of great importance that the attention of the premedical and medical students be directed to sources.

Viktor Hamburger

Washington University

Die Optik in der Feinmesstechnik. (Optics in precision measurements practice.) Kurt Räntsch. Munich, Germany: Carl Hanser, 1949. Pp. 317. (Illustrated.) Cardboard: 27 DM; clothbound: 30 DM.

Here is a valuable contribution to that division of optical engineering which deals with the use of optical instruments for precision measurements. The first third of the book treats in a very thorough manner those principles of geometrical and physical optics which are necessary to provide a background for the intelligent use of precision optical measuring equipment. The paraxial imaging properties of lenses and mirrors are developed systematically. Considerable attention is devoted to apertures and pupils, and to the transmission of flux through a system. The short section on physical optics covers pertinent topics such as interference and diffraction, resolving power, and light losses by reflection. Possibly some readers would have welcomed a brief mention of polarized light, and of optical interference films for antireflection and beam-dividing purposes. There is a convenient eight-page collection of all mathematical formulas at the end of the book for quick reference.

The remaining two-thirds of the book takes up optical instruments. It is replete with good photographs of an

astonishing variety of products of the firms of Ernst Leitz and Carl Zeiss. The author, who is a member of the Zeiss organization, explains that this limitation to German manufacturers was necessary under present conditions in order not to delay publication. It is to be hoped that his intention to add instruments of other origin in future editions will materialize. The pictorial representation of the subject matter has been carried out so carefully and completely that it should serve to compensate the language difficulties which some may have, the book being, of course, in German. Measuring microscopes, collimators, contour projectors, comparators, gage block interferometers, and apparently all such instruments as would be expected in a text of this kind have found their place. One wonders if industry really makes full use of the potentialities of all this equipment. Finally there is a noteworthy section on surface profile determinations, with comparative photographic documentation of results using the profile microscope, the quenched total reflection microscope, and the interference microscope.

The book will serve the student as a reference or even as a text, and it will provide the instrument maker or process engineer with a guide to the solution of many problems in precision measurements.

A. F. TURNER

Bausch & Lomb Optical Company

Trace elements in food. G. W. Monier-Williams. New York: John Wiley, 1949. Pp. viii + 511. $6.00.

The title *Trace elements* has a variety of connotations for different groups. The nutritionist has in mind primarily iron, iodine, copper, manganese, cobalt and zinc. Agriculturists add boron to this group and dentists add fluorine. The toxicologist, while not unmindful of a possible excess of some of the elements named, centers his main interest on the remaining ones. But the health officer and the food chemist must deal with the gamut of elements, since all are encountered in foods, in more or less quantity, sometime or other.

Monier-Williams' *Trace elements in foods* is written primarily from the standpoint of the food chemist. There are 28 chapters, each dealing with a single element except for the last few chapters, which cover several closely related elements. The book is primarily an abstract of the papers to which it refers—about 1,600 in all. In the case of each element half of the discussion deals with the source and amounts in foods, nature or condition of occurrence, function if any, nutritional requirement, availability, assimilability, tolerance, toxicity, retention, excretion etc. The other half of the discussion is devoted to methods of analysis. Its thoroughness in this field should make it a useful book for every food chemist.

The author discusses briefly the philosophy of regulatory measures. He states: "Agreement [between what is clearly toxic and what is undoubtedly harmless] is not likely to be reached without far more complete knowledge of human physiology than we possess at present. Meanwhile, any preference must be on the side of the consumer." But he weakens that statement by another. "It can be urged that where exhaustive animal experiments have shown no sign of injury of any kind, there is no reason to fear any adverse effect on man and no justification for imposing limits and prohibitions which may harass traders and impede progress without conferring any apparent benefit on the consumer." The different reactions of different species and the various susceptibilities of individuals, particularly invalids, within a species justify greater caution than this implies. The "harassing of traders and impeding of progress" have often been overemphasized. One can cite the former wide use of sodium benzoate in ketchup. Now it is outlawed by the federal definition and standard that industry itself largely formulated. Preservatives in milk, a most perishable food, were outlawed when knowledge was very limited as to what could be accomplished by a combination of pasteurization and refrigeration, and facilities for applying them were even more limited. This prohibition was a challenge to the dairy industry. Today the distribution of milk is on a very high level indeed. Abolition of the use of the violently reactive chemicals now being used in the flour and bread industries might be expected to have an equally salutary effect.

At least part of the medical profession was confident that lithium chloride could be used as a seasoning agent to supplant sodium chloride for patients requiring a low sodium intake. Not a single voice was raised against it until a few deaths were reported. Lithium at one time was regarded as an important ingredient of certain so-called health waters. Incidentally, Monier-Williams' discussion of lithium is very brief. The episode mentioned occurred too recently for him to have included it.

Some other omissions are less easily excused. The author states that a tree may be fed manganese through a hole bored in the trunk. He fails to point out the more and more common practice of feeding plants through their leaves. Important commercial applications of the technique are spraying pineapples with iron and citrus trees with manganese, copper, and zinc.

Through an error, the author states that normally 1¼ to 1¾ lb of tin is used to coat a base box of steel in making tinplate. In this country 3 percent of hot-dipped plate contains 1¼ lb and 97 percent contains only 1.25 lb. The ability of the canning industry thus to spread the limited supply of tin during the war is what made it possible to keep up the supplies of food to both civilians and the military forces.

E. F. KOHMAN

Campbell Soup Company

NEWS and Notes

H. C. Dudley, head of the Biochemistry Division of the Naval Medical Research Institute, Bethesda, Maryland, has been appointed head of the newly organized Allied Medical Sciences Section, Medical Service Corps of the Navy.

U. S. scientists who have been invited by the Paris Centre National de la Recherche Scientifique to attend the colloquium on astronomical constants to be held in Paris, March 27-April 1, 1950, are **G. M. Clemence,** U. S. Navy Observatory, Washington 25, D. C., and **Dirk Brouwer,** of the Yale University Observatory.

William McDowell Hammon, assistant director of the Hooper Foundation, University of California, has been appointed professor and head of the Department of Epidemiology at the Graduate School of Public Health, University of Pittsburgh. In this position Dr. Hammon will also be responsible for the instructional and research interests of the school in the field of microbiology. His appointment is effective February 1, 1950.

William E. Mahin, director of research at Armour Research Foundation of Illinois Institute of Technology, has been appointed a member of the National Research Council for a three-year period ending June 30, 1952. Dr. Mahin will represent the American Society for Metals.

D. A. Fraser, lecturer in botany, University of Alberta, has been appointed forest ecologist, Forest Insect Laboratory, Dominion Department of Agriculture, Sault Ste. Marie, Ontario.

Eric Ogden, formerly of the University of Texas, Medical Branch, Galveston, has been appointed professor and chairman of the Department of Physiology at the College of Medicine, Ohio State University.

The Atomic Energy Commission has appointed two new branch chiefs to its Washington headquarters staff. They are **Walter D. Claus,** former head of the Physical-Chemical Division of the Pabst Research Laboratories, Milwaukee, as chief of the Biophysics Branch, Division of Biology and Medicine, and **Joseph B. Platt,** former associate professor of physics, University of Rochester, as chief of the Physics Branch, Division of Research. Dr. Claus replaces **Lauriston S. Taylor,** who has been on loan from the National Bureau of Standards. Dr. Taylor will return to the position of chief of the X ray Section and will act as consultant to the AEC on radiation matters.

Hsien Wu, visiting scholar, College of Physicians and Surgeons, Columbia University, has accepted a position as visiting professor of biochemistry at the Medical College of Alabama, Birmingham. Dr. Wu was professor of biochemistry at Peiping Medical College from 1928 to 1941, and, in 1944, director of the Nutrition Institute, Ministry of Health, China.

Paul E. Clark, physical chemist and former head of the Department of Chemistry at Washington and Jefferson College, Washington, Pennsylvania, has accepted a position as technical reports writer at the Applied Physics Laboratory of Johns Hopkins University, Silver Spring, Maryland.

Visitors to U. S.

Recent visitors at the Plant Industry Station at Beltsville, Maryland were **Sir William G. Ogg,** director of the Rothamsted Experimental Station in England, and **C. H. Edelman,** director of the Soil Survey in Holland and president of the International Congress of Soil Science.

Recent visitors at the Department of Agriculture were **Gregorio Rosenberg M.,** head of horticultural research, Ministry of Agriculture, Santiago, Chile; **René L. Ambroise,** chief of the Soil Conservation Service, Haitian Department of Agriculture; and **Julio Peña,** professor of chemistry, Central University of Quito, Ecuador.

Visitors at the National Bureau of Standards September 19–30 included: **J. F. Allen,** professor of physics, St. Andrew's College, Scotland; **K. R. Atkins,** physicist, Cambridge University, England; **Axel Bernstein,** metallurgist, the Sandvik Steel Works, Sandviken, Sweden; **A. Fogg,** director, Motor Industry Research Association, Brentford, England; **W. D. Forrester,** field engineer, Canadian Geodetic Survey, Ottawa; **Felix A. Galavis S.,** chief of the Geological Laboratory, Ministeriode Fomento, Caracas, Venezuela; **Sir Charles Goodeve,** director, British Iron and Steel Research Society, London; **A. Goodewaagon,** chemical engineer, Donda, Netherlands; **H. O. Hartley,** lecturer in statistics, University of London, London; **Francis M. Henderson,** senior design engineer, Dominion Physical Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand; **P. Lainé,** physicist at the Bellevue Laboratory, France; **J. M. Los,** physicist, National Research Council of Canada; **M. Meissner,** professor of physics, Technical Institute, Munich; **F. E. Simon, K. Mendelssohn,** and **A. H. Cook,** professors of physics at the University of Oxford, England; **D. S. Montgomery,** Canadian Bureau of Mines, Ottawa, Canada; **J. Singer,** director, Central Organization for Applied Scientific Research for the Netherlands, the Hague; **Hilding Slatis,** professor, Nobel Institute for Physics, Stockholm; **R. A. Smith,** physicist with the British Ministry of Supply, London; **Serge Staub,** sugar technologist, Department of Agriculture, Great Britain, Reduit, Mauritus Island; and **F. W. Wood,** in charge of Watheroo Ionospheric Observatory, Department of Mineral Resources, Melbourne, Australia.

Ryokichi Sagane, professor of physics at Tokyo Imperial University, will spend six months as visiting professor at the Institute for Atomic Research, Iowa State College, beginning January 1. Dr. Sagane will deliver a series of lectures and confer with staff members associated with the synchrotron.

Grants and Awards

The Federal Security Agency has announced the distribution of $3,250,000 among nine teaching institutions to assist in construction of cancer research laboratories. Recipients are: *University of Minnesota,* $200,000 for two floors of clinical research at the Mayo Memorial Medical Center; *University of Chicago,* $240,000 to aid construction of the seven-story Goldblatt Memorial Hospital for Cancer Research; *New England Deaconess Hospital,* Boston, $85,000 for two floors adjoining the New England Deaconess Cancer Institute; *University of Kansas,* $200,000 for one wing of a two-story building for laboratory and clinical research at the University Medical Center; *Johns Hopkins University,* Baltimore, $750,000 to aid construction of cancer research facilities; *St. Louis University,* $625,000 for a new clinical research building at the University Medical School; *University of California,* Los Angeles, $700,000 for a wing of the medical school; *University of Pennsylvania,* $200,000 for a one-half-floor laboratory; and *Memorial Hospital for Cancer and Allied Diseases,* New York, $250,000, for an additional floor for an experimental surgical laboratory.

The John Fritz medal for 1949, for "scientific or industrial achievement," has been presented by the Engineering Societies to Walter Hull Aldridge, president of the Texas Gulf Sulphur Company. Mr. Aldridge was cited for his contribution to the mineral production of the U. S. and Canada. The societies have also announced that the 1950 **Daniel Guggenheim Medal** for achievement in aeronautics will be given to Edward P. Warner, president of the Interim Council of Provisional International Civil Aviation Organization.

Carnegie Institute of Technology has awarded $1,000 graduate fellowships in engineering to René Saul M., Mexican electrical engineer formerly associated with RCA Victor Mexicana, and Edilberto Vega P., Peruvian civil engineer who has worked with the Department of Public Works and the Division of Sanitary Projects in Lima. The fellowships are granted annually to Latin Americans in the interest of intercontinental unity.

The National Institutes of Health have awarded to **John C. Krantz, Jr.,** professor of pharmacology, School of Medicine, University of Maryland, a grant of $6,500 for study of the mechanism of the action of drugs on the cardiovascular system.

Grants totaling $8,614,737, to be administered by the **National Heart Institute of the Public Health Service,** have been awarded 85 medical schools and research institutions in 34 states and the District of Columbia. The funds will be used for heart research, expanded programs of heart teaching in medical schools, and for building additional heart research laboratories throughout the country. The grants are in addition to those awarded in July (amounting to $1,200,000 for continuing research projects already under way) and provide a total of nearly ten million dollars in federal funds appropriated during the fiscal year ending June 30, 1950 to combat heart disease. The categories of the grants are: $2,053,310 for 189 research investigations in 66 nonfederal institutions in 28 states and the District of Columbia; $671,032 for improving and expanding cardiovascular teaching in 46 medical schools in 28 states and the District; and $5,890,395 for providing research laboratory facilities for study of heart diseases in 22 nonfederal institutions.

The Atomic Energy Commission Division of Biology and Medicine has approved 18 research proposals in biology and medicine during the past three months. The institutions and their research projects are: Massachusetts General Hospital—*William M. Sweet* and *Bertram Selverstone,* for the use of phosphorus-32 in the precise localization of brain tumors; University of Oregon Medical School—*Edward E. West,* for a study of labeled acetic acid and ethanol in relation to fat metabolism; University of Tennessee—*E. F. Williams,* for research in pathology, physiology, and chemistry; Presbyterian Hospital of the City of Chicago—*R. Gordon Gould,* for study of the mechanism of CO_2 fixation; Peter Bent Brigham Hospital—*Francis D. Moore,* for study of intracellular changes in trauma depletion and repair, and biochemical studies in the human being with the aid of isotopes; University of Denver—*Frank M. D'Amour,* for study of the physiologic and pathologic effects of radioactive cobalt; Harvard University—*Thomas H. Ham* and *William B. Castle,* for study of the destruction of red blood cells; Harvard University—*S. P. Hicks,* for study of the effects of radiation upon the development of rat embryos; Syracuse University—*E. L. Lozner,* for study of the defenses against hemorrhages; Washington University, St. Louis—*Frank Dixon,* for investigation of the effects of agents used in the treatment of cancer, and study of x rays and nitrogen mustards on the immunologic response of experimental animals; University of Illinois—*A. C. Ivy,* for irradiation of gastric mucosa by intragastric instillation of radioactive isotopes; Iowa State College—*S. Aranoff,* for study of the metabolism and physiology of roots, and *R. E. Sealock,* for study of combined biochemical and physiological actions of throsine and vitamin B_{12}; Agricultural and Mechanical College of Texas—*Raymond Reiser* and *Kenneth Kuiken,* for study of the metabolism of glycerines; University of Pennsylvania—*D. Wright Wilson,* for study of the synthesis of isotopic carbon compounds used in biochemistry; University of Chicago—*E. M. K. Geiling,* for the study of biosynthesis of radioactive drug compounds; University of Wisconsin Experiment Station—*B. W. Burris* and *P. W. Wilson,* for studies of biological nitrogen fixation with isotopic tracers, and study of the metabolism of organic acids in higher plants and microorganisms; University of Washington—*F. W. Church* and *Raymond Allen,* for meteorological studies; *Robert G. Fleagle,* for meteorological studies.

Negotiations leading to the award of contracts covering the newly approved research projects are now under way and contracts will be awarded by the AEC operations office nearest the institution conducting the research.

The award of contracts to the institutions involved will bring to a total

of 150 the number of AEC-supported research projects being carried on in medicine, biology, cancer studies, and biophysics at universities, hospitals, and research centers. Approximately five million dollars has been allocated by the AEC for support of such research in nongovernment agencies during the fiscal year 1950.

Meetings and Elections

A series of 12 weekly lectures on the **Psychology of Emotional Growth** began on October 11 at Cooper Union in New York City. The series is open to the public without charge or seat reservations.

The National Academy of Sciences will hold its autumn meeting at the University of Rochester, New York, October 24–26. Abstracts of papers presented will be published in the October 28 issue of *Science*.

The Engineers' Council for Professional Development will hold its 17th annual meeting at the Edgewater Beach Hotel in Chicago October 28–29. Programs may be obtained in advance of the meeting by writing to George G. Lamb, Technological Institute, Northwestern University, Evanston, Illinois.

The 450th meeting of the **American Mathematical Society** will be held at Columbia University on October 29. R. H. Fox, of Princeton University, will deliver an address on covering spaces.

The Fourth Annual Congress on Horticulture will be held October 30–November 1, at the Essex House, New York City. The congress will include a symposium and round table on color, preliminary to the publication of a 2,000-color standard chart by the Commission on Testing and Reporting of the American Horticultural Council. Further details may be obtained from R. Milton Carleton, 601 West Jackson Boulevard, Chicago 6.

The **Gulf and Caribbean Fisheries Institute** will hold its second annual meeting November 15–18, at the Robert Richter Hotel, Miami Beach, Florida. Papers will be presented by delegates from Cuba, Martinique, and other Caribbean countries, as well as from the southeastern states of the U. S.

ACS at Atlantic City. One thousand sixty-five papers—by far the largest number in the history of the American Chemical Society—were presented at the society's 116th national meeting, held in Atlantic City, New Jersey, September 18–23, with 8,232 chemists and chemical engineers participating. Nineteen professional divisions of the society held 151 technical sessions, at which advances in virtually every chemical field from agriculture and biology to petroleum and rubber were reported.

An optimistic keynote was provided by Arthur B. Lamb, of Harvard University, retiring editor of the *Journal of the American Chemical Society*, who received the Priestley Medal at a general assembly in the Atlantic City Convention Hall. Science, whose revolutionary progress has plunged man into the maelstrom of social upheaval, will lead him safely through into a better world than he has ever known, Professor Lamb declared. Despite the grave problems now confronting civilization, there is no need to be "unduly depressed," he said, because "fundamentally, and for the long pull, mankind's prospects have never been brighter."

An appeal to private industry to insure its own continuing progress and the future welfare of the nation by creating a $75,000,000-a-year foundation for the support of basic research was made by the society's president, Linus Pauling, of the California Institute of Technology. Such a foundation would not eliminate the need of federal aid for research, but it would avert the menace of bureaucratic domination of scientific study and thus help preserve the American system of free private enterprise, Professor Pauling said.

The names of seven 1950 award recipients were announced by Dr. Pauling. They are:

Garvan Medal—Pauline Beery Mack, director, Ellen H. Richards Institute of Research in Textiles and Nutrition, Pennsylvania State College; *American Chemical Society Award in Pure Chemistry* (financed by Alpha Chi Sigma)—Verner Schomaker, California Institute of Technology; *Precision Scientific Company Award in Petroleum Chemistry*—Kenneth S. Pitzer, (University of California), director of research, Atomic Energy Commission; *Eli Lilly and Company Award in Biological Chemistry*—William Shive, University of Texas; *Fritzsche Award in Essential Oils*—A. J. Haagen-Smit, California Institute of Technology; *Fisher Award in Analytical Chemistry*—Isaac M. Kolthoff, University of Minnesota; *Paul-Lewis Laboratories Award in Enzyme Chemistry*—Britton Chance, director, Johnson Foundation, University of Pennsylvania. The awards will be presented at the society's spring meeting, which will be divided into three sessions at Houston, Philadelphia, and Detroit.

First announcement in the U. S. of the decisions of the International Union of Chemistry on the names of plutonium and 13 other elements was made at the ACS meeting. Alexander Silverman, University of Pittsburgh, American representative on the union's Commission on Inorganic Nomenclature, who had just returned from the union's 15th conference in Amsterdam, reported that these names had been accepted for the eight new elements discovered during World War II: technetium (43), promethium (61), astatine (85), francium (87), neptunium (93), plutonium (94), americium (95), and curium (96). He also listed the following rulings on names of older elements: wolfram instead of tungsten for number 74; niobium instead of columbium for 41, beryllium rather than glucinium for 4, hafnium for 72, lutetium rather than lutecium for 71, and protactininum rather than protoactinium for 91.

Widespread interest was aroused by a symposium on "Security Clearance and the Scientist," sponsored by the society's Industrial and Engineering Division, at which Colonel E. M. Tally, Jr., chief of the Munitions Board's Office of Manpower, announced that the Department of Defense is establishing a central security file to make the entire clearance system simpler and to eliminate much of the duplication existing under separate Army, Navy, and Air Force procedures.

Discovery of a simple, low-cost method for the mass-scale production of essential amino acids for safe intravenous feeding was reported by Jesse P. Greenstein, of the National Cancer Institute.

Recent findings in the biochemical study of diabetes, achievements in the flameproofing of textiles, the varied industrial uses forecast for titanium, and developments in the fields of paints, plastics, rubber, and insecticides were among the other subjects discussed at the meeting.

WALTER J. MURPHY

The Mt. Desert Island Biological Laboratory is planning to issue early in 1950 a *Bulletin Number* covering the years 1941–49, and a list of papers resulting from work at the laboratory from 1929 through 1949. All contributors are asked to submit titles of published papers or a sentence description of unpublished work by *December 1* to J. Wendell Burger, Director, Trinity College, Hartford 6, Connecticut.

The Philadelphia Section of the American Chemical Society will sponsor again this fall special non-credit evening courses in physiology for chemists and recent developments in colloid chemistry. The lectures will be held at the Philadelphia College of Pharmacy and Science, 43rd and Kingsessing Avenue, Philadelphia. Attendance is not limited to members of the American Chemical Society. Further information may be obtained from E. J. Rosenbaum, Sun Oil Company, Norwood, Pennsylvania, chairman of the Philadelphia Section's Chemical Education Committee.

The New York Academy of Medicine has donated 12,000 volumes to the Southwestern Medical College in Dallas, Texas. These volumes are part of a gift to the academy of 20,000 volumes from the New York Public Library, representing its original collection of medical books accumulated during the 19th century. The academy is adding the remaining 8,000, including valuable foreign medical publications and issues of unusual journals, to the 260,000 volumes of its present library.

The National Bureau of Standards has just published its annual report summarizing investigations in the physical sciences carried on at the bureau during 1948. Activities were conducted by 14 divisions, concerned with electronics, applied mathematics, atomic and molecular physics, radio propagation, electricity and optics, metrology, heat and power, chemistry, mechanics, organic and fibrous materials, metallurgy, mineral products, building technology, and commodity standards. Of the projects carried out by approximately 100 sections within these divisions, those of greatest general interest and widest application have been selected for description. The 272-page illustrated booklet is available from the U. S. Government Printing Office, Washington 25, D. C.

The New York Academy of Sciences, now located at Central Park West and 79th Street, New York City, will occupy new headquarters at 2 East 63rd Street after January 1. The new residence is the million-dollar gift of Norman B. Woolworth. The academy is conducting a campaign for one million dollars, half to be used for alteration, equipment, and maintenance of its new home and half to be set aside for an extension of activities.

Recently Received—

Medical Mission to Greece and Italy, April 15–June 7, 1948. Abridged report. Unitarian Service Committee, Inc., 9 Park Street, Boston 8.

Sixth Report of the Biological Bureau, Quebec, 1948. Game and Fisheries Department, Quebec, Canada.

The Navajo Meteorite. Sharat Kumar Roy and Robert Kriss Wyant. Geological Series of Field Museum of Natural History, Chicago. Vol. VII, No. 8. 30¢.

List of Publications, Department of Terrestrial Magnetism, 1948. Carnegie Institution of Washington, Washington 15, D. C.

Handbook on Florida Termites. E. Morton Miller. Technical Series, University of Miami Press, Coral Gables, Florida.

Proceedings of the First Annual Northern California Research Conference, January, 1949. Stanford Research Institute, Stanford, California. $2.00.

Un diagramme nouveau a quatre coordonnees. P. Lenk-Chevitch. Extract from technical bulletin of l'Union des Ingenieurs sortis dee Ecoles Speciales de l'Universite de Louvain, Brussels.

Isotopes, Catalogue and Price List No. 3, July 1949. Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tennessee.

Ecological Crop Geography of Germany and Its Agro-Climatic Analogues in North America. M. Y. Nuttonson. International Agro-Climatological Series, Study No. 8, 1949. On request from American Institute of Crop Ecology, P.O. Box 1022, Washington, D. C.

A Collection of Fishes from Talara, Peru. Samuel F. Hildebrand and Otis Barton. Miscellaneous Collections, Vol. III, No. 10, Publ. 3986, Smithsonian Institution, Washington, D. C.

Make Plans for—

Pacific Chemical Exposition and **Pacific Industrial Conferences,** sponsored by the American Chemical Society, November 1–5, Civic Auditorium, San Francisco.

Special Libraries Association, council meeting, November 3–5, Hotel Statler, New York City.

American Association of Blood Banks, November 3–5, Seattle, Washington.

Society of Rheology, 20th annual meeting, November 4–5, Hotel New Yorker, New York City.

American Society for the Study of Arteriosclerosis, November 5–7, Hotel Knickerbocker, Chicago.

American Society of Tropical Medicine, meeting conjointly with the **National Malaria Society** and **American Academy of Tropical Medicine,** November 6–9, Memphis, Tennessee.